# NUTRITION : THE NEWER DIAGNOSTIC METHODS

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PROCEEDINGS OF THE ROUND TABLE ON

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# PREFACE

Despite the improvement of dietary habits in the United States, several lines of evidence point to the prevalence of impaired nutrition among the population. Nutrition is a bodily process; therefore detection of any derangement, with its symptoms, signs, and underlying pathology, constitutes a medical problem. It has become an increasingly difficult problem with the recognition of the latent state as a definite though now invisible zone in nutritional conditions, and the need for objective methods sufficiently sensitive to be used for early diagnosis has become more pressing than ever. Fortunately research in the laboratories has kept pace with the consciousness of this need, by bringing out not a few of the necessary biochemical and physiological procedures.

These tests have been developed and perfected as separate units with no planned liaison between them. Taken individually, each is a valuable implement; considered collectively, with full recognition of gaps to be filled and improvements yet to come, they bulk even larger in their promise of usefulness in diagnosis. Unquestionably timely as a topic in view of the need to define more precisely the problem of malnutrition, the tests seemed sufficiently mature to warrant fuller exploration of their potentialities. Accordingly, clinical and laboratory investigators gathered, under the auspices of the Milbank Memorial Fund, to take account of the available procedures; to deliberate, with reference to both technical points and broad outlines, on the applicability of each; and to envisage all in their entirety.

The present volume contains a full account of the round table on nutrition at the Annual Conference. Its main topics introduce much that is recent knowledge, some available only in widely scattered journals and some previously unpublished; the discussions are such as usually do not find their way into print. There is another consideration. The participants in the Conference being necessarily limited in number represent only a frac-

tion of those who might wish to scan the record. Accordingly, this monograph brings together the material under one cover so that it may reach a wider circle in a convenient form.

Once again, we would express our appreciation to the participants who prepared their reports and remarks for publication. We are likewise grateful to the editors of several journals who courteously permitted the use of certain material originally appearing on their pages.

F. G. Boudreau
H. D. Kruse

# THE MEDICAL ASPECTS OF PROBLEMS OF NUTRITION

A. GRAEME MITCHELL[1]

THIS conference on nutrition is one of interest alike to the physiologist, the biochemist, and the clinician; most certainly to the clinician who is, as usual, awaiting the results and conclusions from the work of the other two. He must do this with a patience which is sometimes hard to muster, since he is daily faced with nutrition problems which in their acute phases are readily apparent, and he wonders how often he fails to recognize them in their more subtle forms. Even when the problems are apparent, it is not always clear what to do about them.

As a pediatrist it stimulates me to realize how much of laboratory and clinical research is applicable to early life. On the agenda of this conference, for example, are a number of subjects related to nutrition, which is certainly of fundamental importance during that period of life when growth is occurring. The baleful effects of undernutrition or malnutrition are perhaps of greater concern during formative years than after growth has been completed, significant as is this latter period. For this reason I am sometimes tempted to believe that pediatrics is the most important aspect of clinical medicine; or, at least, that children present a more urgent problem than adults.

Occupying a conspicuous part on the program are problems of diagnosis, with presentations dealing with the determination of deficiency diseases in their early stage, to which are sometimes applied the terms, "sub-clinical" and "sub-morbid." I wonder if these are satisfactory designations? In reality the initial manifestations of deficiency diseases are both clinical and morbid, and they could be termed "sub" only because in many instances there are not as yet the means of recognizing them. Most of us are capable of diagnosing scurvy, rickets, and pellagra when they rise

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up and smite us, as it were. It is obvious, however, that no one of these deficiency states bursts fully developed upon the scene; their very nature proves that the final, severe manifestations must have been preceded by latent periods—latent only because they were unrecognized. The symptoms associated with these early deficiency states are, to say the least, obscure. Accordingly, the need is recognized for more sensitive means of determining these primary states of deficiency.

The deficiencies which affect the human are not due alone to lack of vitamins. There may, for example, be improper supply and balance of minerals and the program does not neglect this phase of the subject, nor that of other types of disturbance as of the blood-protein which are a consequence of insufficient supply of protein.

This meeting cannot hope to answer all the questions which could be propounded. Much work remains to be done in the evolution of tests for nutritive disorders, in the determination of the constituents of optimum nutrition and in its attainment by the individual. Nevertheless, on such matters reports of progress can be made and problems can be more sharply defined. It is of significance, therefore, that a meeting has been called for the purpose of discussing such topics. As a clinician I am impressed by the fundamental fact back of this meeting; namely, the search for the optimum. The world is full of obviously sick and disabled persons, but there are many millions who, while still functioning, could carry on with greater ease and efficiency if they were brought to optimum health. The search must be first to determine the limit of this optimum and then to develop methods to secure it. The latter may be relatively simple when the former is attained.

# THE USE OF ROENTGENOLOGY IN ASSESSING THE NUTRITIONAL STATE AND IN DETECTING CERTAIN DEFICIENCY DISEASES

T. WINGATE TODD[1]

AT a conference on nutrition and public health, even with the title assigned to this address, it may be taken for granted that my task is not to emphasize those early objective indications of disease, the setting of the stage for that last grim and tragic episode when "strength must die defying death." I take it rather that my burden is the presentation of something far more fundamental, something that underlies discussion of independence in a girl, of courage in a boy, of adequate opportunity, of helpful social environment, of education, recreation, the inculcation of self-discipline and the choice of friends, namely, the right of every growing boy and girl to perfection of structure, to the efficiency of function and adaptation which that implies. So I shall spend no time on scurvy, rickets, and the like, but accept my responsibility squarely to seek my problems on that debatable ground which slopes gently downward from the sun-warmed peak of health.

The subject almost automatically subdivides itself under four captions, namely, the effectiveness of the general bodily growth pattern, the sufficiency of the mineral reserve, the evidence of unrecognized acute disturbances of health, and the functional integrity of the tissues of adaptive response, namely, the connective tissues, the alimentary tract, and the locomotor system which, respectively, determine our resistance, our nutrition, and our joy of action. On each of these subjects roentgenographic appraisal has much that is useful to offer.

I speak this with confidence because it has been my good fortune to have associated with me in this work Miss W. Kuenzel whose experience, technical skill, and power of training others in

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roentgenographic method are those possessed only by genius and doubtless would not have been developed even here had not the opportunity offered by the enormous number of roentgenographic studies made by the Associated Foundations inspired her with the realization that here was a fertile field of research untouched till now. What therefore I am able to claim in later paragraphs as the result of our common experience I owe to this unique association.

We take then the first caption, effectiveness of the bodily growth pattern. To most of us this merely means increase in weight and stature. But weight being a function of the soft tissues and stature a corollary of the bones I shall deal with the latter now, the former later on. If growth were indeed a mere matter of inches the outlook for education would be poor indeed. Education deals with organic substances not with dimensions. It is not the growing body but the maturing body on which education may indelibly impress its patterns of thought and action. The child who merely grows is dull, uninteresting as a vegetable, but the child who is growing up, maturing in his outlook and his inner discipline, striving after manly independence or developing in womanly charm, that child captivates us by evident potentiality and citizenship value.

I want to make very clear this distinction between growing and growing up. That the two phenomena fuse themselves in childhood does not minimize their distinct character. A boy does not begin to grow a beard when he reaches a certain stature nor a girl menstruate at a definite age. Both the growing of a beard and the occurrence of the menarche are examples of biological change, symbols of the maturation process which is not dependent on growth and is wholly unrelated to sidereal time. The question is whether we can define the stage and measure the progress of biological change (maturation) as we can of stature (growth) and of age (time).

That the measurement of progress in maturation by roentgenography is indeed practicable is now abundantly clear. It has been demonstrated in detail for man (8) and for the guinea pig (13). It has been broadly sketched for ungulates (9) and for

rodents (10). It has moreover been shown that while there is a relationship to sidereal time of this progress in maturation typical for each mammalian species, that relationship may be retarded by endocrine disturbance (12) or nutritional defect (6). The retardation shown by any child or growing mammal is indeed a measure of disturbance. Minor degrees of disturbance are not uncommon and may be corrected in time provided constitutional health is restored and maintained. The period in which disturbances of this nature are most commonly corrected by Nature is the period of puberty when speed of maturation is quickened beyond the rate characteristic of earlier childhood.

Maturation differs from growth in dimension in its greater susceptibility to correction. We all grow up to uniformity of adult maturation. We do not all grow to uniformity in adult stature. Standards of maturation are identical for all social groups, for all degrees of nutrition, for all human stocks and, if precise relationships to sidereal time be set aside, for both sexes. Standards of stature on the contrary are influenced by all these several factors.

So much for the effectiveness of the general bodily growth pattern.

For records of the sufficiency of the mineral reserve we must appraise the roentgenogram in another manner.

Ninety-seven to 99 per cent of the mineral of the body is stored in the skeleton which undergoes metabolism at so rapid a rate that 30 per cent of the phosphorus stored in the skeleton is eliminated (in the rat) by the end of 20 days (1). Lachmann and Whelan (3) recently made some roentgenographic studies on bones before and after they had been treated with acid to dissolve out part of the mineral. These authors came to the conclusion that a change in between 7 and 22 per cent of the mineral is evident roentgenographically to the naked eye. We do not believe that crude solution of mineral by so artificial a means is a convincing test. Miss Hagemeyer and I (11) have therefore made roentgenographic studies of the skeletons of cadavers including the second phalanx of the little finger, a bone of which we then measured the volume before ashing at low temperature, so that

we obtained the weight of mineral per unit volume of bone. Putting our analyses to the test of inspection we found that we could readily differentiate a change of 25 per cent of total mineral per unit volume. It is not enough, however, to make a simple statement of appraisal. The change must be stated in terms of bone structure. Cancellous tissue of bone presents a close network of fine trabeculae the interstices of which are filled with a gray sheen of labile mineral. Depletion of mineral reduces the gray sheen and leaves the trabecular network standing stark and vivid in its contrast with the now clear interstices. Further depletion results in actual fragmentation of trabeculae and break down of the network. Restoration of mineral is shown first by thickening of trabeculae with reconstruction of the network and formation of nodes or knots of mineral at trabecular intercrossings like the drops of dew on a spider's web. Finally the gray sheen returns and the trabecular network becomes less distinct.

It goes without saying that we could not identify these changes had we not actually seen them occur in living subjects under repeated observation. Adolescence, pregnancy, and lactation are the periods during which these changes can most readily be identified. The time which must elapse before a roentgenographic discrimination may be confidently made is about 6 weeks. That differences in mineral content can be objectively recorded, that the time necessary for these changes to occur can be specified, and that check observations by chemical analysis on cadavers can be employed as standards, together demonstrate a large and rapid mineral metabolism conformable to the claim made by Chiewitz and Hevesy (1). None but the crudest estimations however can be made on the routine roentgenograms customarily taken, of which the least reliable are those made in cassettes or on the Bucky diaphragm, both being techniques which increase contrast at the expense of faithful reproduction of texture. The only reliable processing for this purpose is a standard exposure and development technique which assures uniform roentgenographic density. This may be facilitated by an aluminum gauge registering steps of metal thickness from 1 to 10 millimeters (5). The gauge of course does not directly assist in the ap-

praisal of mineral content but validates the appraisal in proving by the gauge shadows that the two roentgenograms to be compared have undergone identical processing. The gauge is therefore analogous to but not the counterpart of a colorimeter. Its employment is essential to those who demand precision of determination and whose knowledge of the structural detail of bone is adequate for a proper interpretation of its roentgenographic appearance.

We pass then to the third caption of our study, namely, the evidence of unrecognized acute disturbances of health. It is common knowledge that any illness, injury, or operative procedure temporarily inhibiting growth in a bone in which mineral is being accumulated to meet the demands of growth, results in the formation of a dense line or scar marking the pause in growth (4).

It is not however yet generally understood that minor health disturbances usually mediated through the gastro-intestinal tract result in multiple scorings or finer transverse striations near the growing end of the shaft (7). These striations resemble the lines on watered silk. They are far more temporary than scars and often disappear completely in 6 months. We have described and figured them (6). They are characteristically but not invariably present in the gastro-intestinal phase of active allergy (2), for their presence depends as much on constitutional susceptibility as upon the intensity of the disturbance.

We now take up the last phase of this study and discuss roentgenographic appraisal of the functional integrity of the tissues of adaptive response, connective tissues, locomotor system and alimentary tract.

The betrayal of gastro-intestinal disturbance by the presence of bone scorings has just been discussed and need not be further emphasized. We may pass at once to the appraisal of connective tissue.

On a soft tissue roentgenogram carefully processed in the manner demanded above, the density of subcutaneous and intermuscular connective tissue may be appraised. The density depends upon the relative proportion of tissue hydration. When

this is correct the connective tissues are moderately clear permitting identification of the skin thickness (subcutaneous tissue) and deeper (not superficial) muscular outlines (intermuscular connective tissue). When, however, the connective tissues are waterlogged, their density is increased so that identification of the deeper limit of skin becomes difficult and the deeper outlines of muscles impossible. In place of the clear subcutaneous tissue contrasting with the denser shadows of blood vessels contained therein, a uniform putty-like density obtains. The same putty-like uniform shadow replaces the clear differentiation of muscles in the depths of the limb. This waterlogging, like bone scorings, is characteristic of but not invariably present in active gastrointestinal allergy. Its occurrence is not however confined to that disorder. A temporary increase in body weight accompanied by an increase in density of connective tissue has therefore a connotation very different from that of an increase in weight when the connective tissue shadows remain clear. The latter is evidence of a genuine increase in panniculus, for fatty tissue gives a clear roentgenographic shadow compared with that of a waterlogged tissue. These observations have been checked and substantiated by roentgenographic studies of cadaveric limbs before and after embalming, which invariably increases the density of connective tissue shadows. Intra-articular tissues like the *ligamenta alaria* of the knee betray changes in hydration on the soft tissue roentgenograms precisely as the connective tissues do. In hydrops of a joint resulting from sprain the condition is readily observable roentgenographically by the density of shadow in the joint as well as by the displacement of capsular outline and adjacent muscles. Sprains of the elbow lend themselves peculiarly well to this precision of observation.

Swelling of the subsynovial (periarticular) tissues of the knee reduces the clear rhomboidal area observable on the lateral roentgenogram between patella and *ligamentum patellae* in front and femur and tibia behind. The sharply defined outline which normally characterizes this rhomboid is replaced by a softer shadow of thickened edematous tissue. Sometimes thickened swollen subsynovial tissues and dense swollen *ligamenta alaria*

can be seen to occur together. Sometimes the dense shadow of waterlogged tissue invades even the epiphyseal area.

Tissues presenting these dense shadows, whether they be connective, articular or epiphyseal, are not infrequently the site of "growing" pains, more or less intractable and often mistaken for mild attacks of rheumatism. The pain may be relieved by gentle massage or temporary immobilization in a cast, but, unless the waterlogging disappears, the pain returns on removal of the cast.

Density of muscular shadow is of a different order from density of intermuscular tissue. It is best appraised on the shadow of the thenar musculature seen in the lateral roentgenogram of the hand. In our as yet unpublished studies of athletes and of nonathletes in the Cleveland schools, the contrast between the well-mineralized bones of athletic boys and the poorly mineralized bones of the nonathletes was coupled with a density of muscular shadows in the former and a relative lightness of muscular shadow in the latter. Reduction in muscular density usually accompanies the lapse of vigor in the post-puerperal period and it is also seen in constitutional debility from any cause in childhood. Muscular shadows in infancy are characteristically light.

It has been my aim in this presentation to avoid discussion of those roentgenographic features of deficiency diseases which are common knowledge and to open instead the discussion of a field new to medicine, namely, the use of the roentgenogram in detecting early signs of deviation from normal structure which may be of assistance in assessing the disturbance of health before that disturbance becomes pronounced enough to give clear-cut evidence of disease.

## SUMMARY

Carefully processed roentgenograms made by standard technique, without the use of cassettes or the Bucky diaphragm, developed at 65° F. to a uniform density which differentiates the soft tissues as well as the detailed structure of bone are most helpful in differentiating deviations from normal structure before those deviations have become pronounced enough to give clear-cut evidence of disease.

By these means it is possible to assess the status and progress of the maturation process in the skeleton and thus give information on the general bodily growth pattern.

Reliable appraisal can also be made of the sufficiency of the mineral reserve.

Evidence can be obtained of otherwise unrecognized acute disturbances of health in constitutionally susceptible or debilitated children by means of the scorings (not transverse scars) on the bones.

The functional integrity of the alimentary tract can be indirectly investigated by assuring oneself of the absence of these bone scorings.

Changes in hydration of connective tissue may be recognized both in the subcutaneous and deeper intermuscular areas, in the peri and intra-articular tissues and even in epiphyseal areas.

Comparative studies of the density of muscular shadows give important information in relation to staying power and convalescence.

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## DISCUSSION

Dr. Harry Bakwin: Dr. Todd has mentioned the susceptibility of the growing bone to environmental influence and this is an aspect of the subject under discussion which I should like to stress.

It is not customary to think of bone as a labile structure. The anthropologist generally looks upon skeletal configuration as relatively fixed and uses it as an index of race differentiation. Nevertheless, it is possible to show that the form of the skeleton can be readily changed, during infancy at least, by dietary manipulation.

Infants from a poverty-stricken environment are retarded in both weight and height. Measurement of the transverse diameters shows that these are more retarded in growth than are the cephalocaudal dimensions so that infants from a poverty-stricken environment are as a group more "linear"—to use the word introduced by Stockard—than supervised infants from a better economic environment. That this change is due to an environmental influence may be shown by taking a group of infants from a poverty-stricken environment and supervising them from birth. Their growth and body dimensions will then be found to approximate almost exactly the bony configuration of infants from the better economic environment.

The skeletal changes which occur in simple undernutrition affect not only the skeletal configuration as a whole but the shape of the individual bones as well. The bones in undernourished children are fairly normal in length but their transverse diameters are reduced.

Furthermore the bony structure is affected. The cortex is thin, the bone more transparent to the X-ray, and the trabeculae distinct and coarse but fewer in number than in the normal bone. Dr. Todd has referred to this condition as demineralization of the bone. It is probably due to an associated deficiency of calcium in the diet rather than to sunlight deficiency since the blood phosphorous and phosphatase are ordinarily normal, only the serum calcium showing moderate reduction. Though the infant's diet consists largely of milk, which contains relatively large amounts of calcium, the intake is often inadequate because of frequent infections in the child which interfere with the intake of food.

Of course, dietary deficiencies other than those due to an inadequacy of the energy-yielding constituents and calcium influence bone growth. The effects of vitamin D deficiency and cevitamic acid deficiency are too well known to require more than mere mention.

There are numerous other conditions which affect the bones during the period of growth. Bone changes are seen in a large proportion of infants with congenital syphilis, an infectious disease. In leukemia, a disease of the blood, bone changes are observed in fully half of the children and bone changes form an important part of the clinical picture of Mediterranean anemia. The influence of climate on bone growth is seen in the case of rickets which is, strictly speaking, a climatic rather than a dietary deficiency disease. The endocrines, particularly the thyroid, also influence the bones, which are short and broad in hypothyroidism.

It is of interest that the bone is not merely an index of the immediate health but of the past health as well. The changes in the epiphyseal centers induced by scurvy are known to persist for years. Transverse lines in the long bones, as evidence of retarded growth, are frequently seen in X-ray plates. The bones in healed rickets are frequently short, thick, strong bones.

In contrast to the susceptibility of bone growth to the environment is the relative stability of the time of ossification of the bony centers, i.e., maturation. In infants who are severely malnourished, one occasionally sees delay in ossification of the carpal centers; but even here the maturation is ordinarily normal. We have found no difference in the ossification of the carpal centers between a poverty-stricken group of infants and a supervised group.

The only agencies known to influence maturation are the endocrines: the thyroid, probably the pituitary, and the adrenal cortex.

Dr. Todd feels that maturation should not be related to chronological age. It is difficult for me to understand what base line one is to use if not chronological age. I wish that he would explain this a little more fully.

In regard to mineralization, we have used the aluminum ladder and have discarded it. I think that simple inspection best detects the index of mineralization but the changes must be quite marked before conclusions can be drawn.

Dr. Harold C. Stuart: I have approached the subject from a somewhat different angle than Dr. Todd. I have tried to think of the use of the roentgenogram as of assistance to the physician in appraising the nutrition of the child. Dr. Todd considered the bones first and progressed outward in his discussion of the various tissues. I think you may be able to follow me just as well if I start with the

skin and progress to the bones, for this is the order in which I have formulated the points I have had in mind.

I should like to emphasize in the first place that Dr. Todd has a great advantage over most of us in that he has seen more X-ray films. He has had a perfectly enormous experience in viewing X-ray films of normal children at different age periods, running into several hundred thousand films. Therefore, to dispute with him as to what one may see in roentgenograms is presumptuous on the part of anyone who has had much less experience.

Dr. Todd has also had the advantage of having had quite a remarkable assistant for a number of years who has been able to repeat technical procedures with great accuracy, and probably to give him much better films with greater regularity than could ordinarily be expected. We have been rather fortunate in this respect in recent years, but I should like to emphasize the fact that we are still encountering great difficulty in getting technically comparable films. This makes it extremely difficult to be certain that differences in shadows which we see at one time or another are real and not due to technical variations. These considerations obviously limit the practical clinical usefulness of roentgenograms in the study of the tissues from a nutritional standpoint.

Let us now consider the subcutaneous tissue. The water content of subcutaneous tissue varies tremendously in different individuals and at different times. I have been trying to visualize these differences in our films, having become interested through Dr. Todd's work.

Quite frequently, one sees a film in which subcutaneous tissue appears very opaque, and in which the child's tissues on direct examination feel waterlogged. One is quite satisfied that the subcutaneous tissue in this child is full of water or has a higher water content, at least, than one would ordinarily expect. There has been a good deal of clinical evidence in the past that the eczematous and so-called allergic child does have greater fluctuations in water content in his subcutaneous tissue than do more normal children.

We see striking changes in weight, that is marked gain or loss of weight, on relatively small changes in diet in these children. But we see the same changes at times in nonallergic children, at least in children whom we are not able to classify as allergic clinically. I do not believe that allergy alone explains these variations in water content. As a matter of fact, Dr. Todd did not express this view in respect to subcutaneous tissue, but he referred to it in connection with the

opacity of the soft tissues of the joints. It seems to me that opacity in shadow over a joint may be due in part to greater density of the subcutaneous tissue, due to higher water content. This may be expected with greater frequency in the allergic child than in other children. We know that the water content of the skin depends in some measure upon the type of diet and can be very considerably altered in other than eczematous children by changes in diet. The important point is that in viewing X-ray films of extremities, we would do well to cultivate the ability to recognize differences in densities of the soft tissues because they are of clinical importance. We have, as clinicians, formed the habit of picking up the subcutaneous tissue and saying whether we think it is dehydrated, whether it is normal, or whether it is edematous; but the film may prove with practice to be an adjunct in this respect.

Turning to a consideration of the muscles, there are enormous differences in the amount of muscle in children of different ages. I think the more we study the amount of muscle, the more we become impressed with the fact that in the developmental pattern, some children are very slow, and others are rapid in respect to the formation of adequate musculature. Dr. Todd has referred to the interpretation of the amount of muscle as seen in X-ray films. Because of the fact that the muscles constitute such an important part of the total body weight, the clinician should keep the amount of muscle in mind when he is attempting to explain variations in weight. He has thought of the lack of adequate weight as being indicative either of a small bony skeleton or of lack of storage of fat and subcutaneous tissues; but has seldom given much thought to the influence of muscles. This leads to faulty judgments respecting nutrition, because muscle size may be an individual or developmental attribute.

Children vary tremendously in the volume of their muscles at given ages as well as at succeeding ages, and if we evaluate this volume, we may be able to explain many of the differences in weight. Attention has been given to skeletal weight as causing variation in total weight, but the bony skeleton does not weigh nearly as much as the muscular system, and therefore cannot have as much effect upon the variations in total weight. Muscle size is somewhat correlated with skeletal size, but there are great differences between muscular individuals and so-called obese individuals. The latter are frequently handicapped by great amounts of soft tissue and inadequate amounts of muscle tissue.

How may we determine the size and amount of muscle in the body? We can feel certain muscle groups, but it is very unsatisfactory to make judgments in that way. We can interpret function; that is, if a child uses his body well, probably he has good muscles. The X-ray does give us an opportunity to visualize the outlines of the muscles and to obtain a clue at least as to their total mass in the body. We should learn to recognize differences in muscle mass while studying X-ray films.

I am under the impression that the development of muscles is more independent of nutrition than the other factors we are considering; that it is part of the individual's developmental pattern to have good muscles or poor muscles. It may be that psychological factors have something to do with this. Possibly, the athlete early in life acquired an interest which helped to develop his muscles through use. Or possibly, the athlete developed an interest in athletics, because he was of the type that develops strong muscles early. The nonathletic child may develop an aversion to athletics, because he knows he cannot perform or will become fatigued owing to relatively slower motor development. Probably, both factors are operative.

The X-ray, in any event, is able to give us some idea of the relative motor development of a child at any given age. The question of whether it is the best evidence cannot be settled here. I might call your attention to the fact that if one can secure accurate 24-hour specimens of urine and measure the creatinine output, one may have a better indication of the mass of muscles in the body than that afforded by X-ray films, because creatinine has been shown to be an indication of active protoplasmic mass. However, that is technically a difficult procedure. It might be more practical in clinical work to view the X-ray film and to compare the findings with clinical judgment as to the amount and quality of these tissues as noted on direct examination.

Now we come to the study of the bones in X-ray films. The extent of osseous development and the pattern of bones may be influenced by nutrition. The size and shapes of bones definitely are influenced by nutrition in the long run, but these aspects are not immediately responsive to inadequacies in diet over shorter periods. Dr. Bakwin has referred to the fact that the infant on an inadequate diet is less retarded in linear growth than in lateral growth. There are other reasons to believe that size and build, and possibly developmental pattern, are influenced by nutrition.

Dr. Todd showed a picture which suggested that the ossification pattern is not very much retarded by nutritional factors, but I think that may depend in some measure upon which factor in the diet is inadequate.

When it comes to differences in the texture of bones, certain of these are clearly related to nutrition and are easily recognized in X-ray films. Marked differences in the structure of the growth zones, and in their density, are commonly recognized. Scorings and lines of interrupted growth and bands of varying densities are also frequently seen in children with marked malnutrition. Some differences in texture may be explained by intrinsic factors and the pattern of the bone formation in the individual. Certainly, many of them may be explained by metabolic differences, independent of diet. But we have in these characteristics the greatest opportunity for diet to show its effects, particularly its adverse effects when it is inadequate. These differences are, or seem to be, largely explainable on the basis of environment, and the environment of bones is to a large extent modified by diet.

We are all familiar with the fact that if a child has infantile paralysis and is put to bed and left there or does not use an extremity over a great many weeks or months, the bones in that extremity lose their normal opacity to X-rays because they lose their calcium content. This occurs even though the child is eating a perfectly adequate diet. So we must not conclude that the finding of demineralized bone is proof that the diet has been lacking in mineral or in vitamins. Normal use is apparently one of the other possible factors.

The signs of qualitative differences in the bones which may be evidences of nutritional influences can be divided into specific and nonspecific categories. We should keep clearly in mind whether we are dealing with specific or nonspecific evidence of faulty nutrition when we see a certain feature in a bone. Specific evidences of vitamin C deficiency and vitamin D deficiency are recognizable in the X-ray film. That is, from a study of the X-ray film alone in a well-marked case, we can say that here is a case of vitamin C deficiency, and here is a case of vitamin D deficiency. We do not have to speculate as to what nutritional element is missing or whether it is nutrition or something else.

I have not put demineralization or lack of calcium in the bone in the specific class. Many of you would perhaps disagree and say that evidence of demineralization in the bones, as recognizable in X-ray

films, is specific for lack of calcium, or at least of one of the factors involved in calcium metabolism. It seems to me that there are several possible causes for variation in the amount of calcium in the bones other than dietary intake. I have considered demineralization in the group of nonspecific evidences, that is, nonspecific with respect to dietary. This does not mean that the finding is not important to recognize and may not have nutritional implications.

The other signs of structural differences that have been referred to—the bands of varying densities, the lines of interrupted growth, and so on—should undoubtedly be considered in the same category of nonspecific evidences. Any one of these may in one instance be due to lack of some element in the diet, and in another be due to some other accompanying condition, such as a disturbance in the endocrine balance, changes in use or activity, the effects of infection, and so on.

In conclusion, it seems to me that before we can decide upon the practical usefulness of X-ray films in detecting disturbed nutritional states, there is need for studying X-ray films much more minutely than we have in the past. We should classify them according to certain findings and compare the groups showing particular characteristics with groups which do not show these. These comparisons should be from the standpoint of clinical histories, dietary histories, and health occurrences and other factors. Only thus can the relationships between what is seen in films and what has actually occurred in life be determined.

There is room for an enormous amount of work along these lines, but for all practical purposes we have a right to believe at the moment that the roentgenogram, if properly taken, can give the physician information which can be of considerable help to him in evaluating the nutritional state of the child at the time the film is taken. Successive X-ray films will at times bring to his attention changes in the child's nutritional state or health before he can recognize them or, at least, be certain of them by physical examination alone.

We are all familiar with the conventional hospital physical examination record which begins, "A well-nourished and well-developed child. . . ." The child in question may or may not be well-nourished, but the statement in the record does not give us much assurance, because we know that it is not based upon adequate evidence. It is a snap opinion as to the nutritional state of the child. We can unquestionably be more positive in our conclusions regarding nu-

tritional status if we study X-ray films in conjunction with the physical examination. I would like to urge that in reading X-ray films of extremities, we form the habit of observing the bone, muscle, and subcutaneous tissue from the standpoint of amount or size, shapes and contours, densities, and markings. For the time being, the conditions observed should be interpreted with caution and in the light of history, physical examination, and laboratory studies.

X-ray films then can be of value in respect to nutrition, because it is possible to recognize qualitative differences in tissues which are directly related to nutrition. We are still in the stage, however, when we should look as intently as we can for signs of possible importance, but should avoid drawing conclusions as to their significance, unless they fall clearly in the category of specific evidences of nutritional deficiency.

Dr. Harry Bakwin: I think we must distinguish between growth and maturation or development. Growth of the bone, i.e., change in size, is readily influenced by the environment. On the other hand, maturation, as far as we know, is not readily affected by the environment.

In hypothyroidism one finds a marked retardation in the ossification of the centers and this can be readily influenced by thyroid therapy. Among normal children, however, carpal ossification cannot be influenced by thyroid administration.

I have just been surveying the bone plates of supervised children from the growth clinic at New York University. There is enormous variability. Many 5-year olds, in every way normal-appearing, show less ossification than normal-appearing 2-year olds.

Dr. Harold C. Stuart: Concerning what I said on that point, it was my intention to classify maturation of the human skeleton as one of the inherent rather than of the dietary manifestations. I agree absolutely that there is a wide variation in the ossification pattern of children who have always been well-fed, according to what we now consider to be an optimum diet. There is such extreme retardation in some of these children that many people might classify them as suffering from a deficiency disease of some specific type. We have never been able to find any evidence that the children we have seen in this group have had any deficiency disease. They are quite normal individuals, but extremely retarded in their pattern of maturation.

We have felt that they were so because of some inherent developmental characteristic.

The point I wanted to make when I referred to the maturity of ossification pattern in relation to nutrition was that there was some evidence to suggest that moderate retardation may be caused by nutritional factors. That may not be true, but I wanted to indicate that the question is still open and that dietary factors may cause variations in pattern over and above the more pronounced intrinsic pattern.

Dr. Carroll E. Palmer: I should like to limit my remarks to one rather broad question, which at the present time I fully recognize as being a somewhat unfair and perhaps premature inquiry. Dr. Todd has indicated that X-ray films are useful in connection with several rather broad fields of interest. In practical public health work, one is continually being confronted with the need for practical diagnostic techniques which may be used on a fairly large scale in the general population. In my own particular field of interest, which is child hygiene, the need is especially great for specific and objective diagnostic tests for disease and for the lesser deviations from health. May I ask Dr. Todd, therefore, for any suggestions regarding how, at the present time, his findings can be put to use in practical public health work. In asking this question, please let me repeat that I fully appreciate the fact that it may be premature to pose such a question.

Dr. T. Wingate Todd: A child grows, but he also grows up. He matures and he shows certain evidence of maturing, and we are very apt to take the sexual evidence, primary and secondary, of maturation as being the chief indicator, and it is, of course, a very good indicator, especially in the adolescent period when these defect make themselves so very apparent.

But every tissue matures, though it matures at a different rate and at a different time in life. An organ matures. As far as the skeleton is concerned, our standards were originally taken, as one would imagine, on the actual chronological age of the child; that is to say we thought years ago that if we took an X-ray of the child, we ought to be able to register the actual, or guess the actual chronological age of that child, and we made some stabs at it.

We made errors, naturally, because, as has come out in this discussion, the rate of growing up differs with different individuals

and in those children such as Dr. Bakwin mentioned, who are definitely hypothyroid, the rate is very slow.

Now are we to regard that as a specific effect of hypothyroidism? I think that as physicians we have always thought in terms of the disease rather than the disturbance. But if we get exactly the same, or if we get a similar condition, namely, slow progress in the maturation of the bone without any evidence of hypothyroidism but, let us say, some really obvious malnutrition, then it is plain that this inhibition is not a specific thing; it is a general feature.

Malnutrition does not mean hunger. It might be indigestible food; it might be inadequate substitution in a substitution diet; it might be a metabolic defect of which the hypothyroidism is only a part.

When we began to realize these things, what we did was to take our very large series of children who are recruited, not from the poverty borderline but from the economically secure families of Cleveland, and make a median in the stage of progress of these bony contours for each year of childhood.

Having obtained a median, we used it as a provisional standard for checking against similar medians derived from other groups of children, institutional children, children actually from the poverty borderline, Negro children, children from other cities.

Dr. Carroll E. Palmer: May I interrupt to ask what the result has been as to median of age?

Dr. T. Wingate Todd: It was originally a median for age, but then we found that that particular stage spread over that age, spread perhaps over 4 years, while within the group of children of a single year roentgenograms could be found representing standards over 4 successive years. You can either take one age and get a dispersion in stages of the maturation, or you can take one stage of maturation and get a dispersion in age. It would obviously be invidious if we were to take a standard, a stage of maturation, and say that it is characteristic of a specific age.

Then we find that in Chicago, let us say, or in New England, with an equally good series of children, the relationship of the median bears a different relationship to chronological age. That is an invidious distinction smacking of embattled cities—"Come to Cleveland. Our children are more mature at a certain age than they are in New England," and so on; that sort of thing.

Hence, we take stages by number and not by chronological age. That is why we have emphasized the stages and not the ages in all this work, stages which can be identified by certain specific features of the bones, which will have, of course, a dispersion over age.

You may get one boy of 10 who has the same maturation as another boy of 8, or you may get a boy of 10 who has the same maturation as another boy of 12. You might say that the boy of 10 who showed the maturation of the boy of 12 was accelerated, and that the boy of 10 who showed the maturation of a boy of 8 was retarded.

It may be that in this boy of 10 whose maturation characteristics, say, are up to our standard for the boy of 12, you have really an individual in whom achievement has more nearly approached capacity than in most people. That is a matter for argument, and I do not know that we can get any farther along those lines at the present time.

The point that is important, and the point that we can agree on, I think, is that there are successive stages in the maturation pattern just as there are successive stages in everything else in the growing up of a child.

I would like to add one further point on that which is a result of our experience which now covers records of all mammals, that these stages, these successive stages, are orderly, and the order is not changed; only the relation to time is changed by any factor which causes a disturbance at all.

It is true, of course, that maturation being an expression in the organism is not a specific thing but a general thing. It expresses itself in the different tissues and in the different organs. But one must not pick out a single organ and imagine that it is necessarily representative of the individual. One may think of the monorchid as retarded. He is not. There is no reason why his maturation should follow the testicle that has not descended any more than the testicle that has descended. In our X-ray maturation record we have never been able to find any of the undescended testicle patients retarded in bodily development.

There are people who grow at one rate and mature at another. All the infantile uteri, usually in long, lean, linear ladies, are examples of retarded maturation in one part, but of course their possessors are not retarded in growth.

Now for the question of whether one can influence maturation. Let us divorce ossification of carpal centers, that is to say the appear-

ance of ossification centers, altogether from the progress in ossification of the contours. I think every one of us has known children who have been singularly retarded in the appearance of ossification and in whom, at about the age of 2 or 3, sheaves of ossification spring up all over the body. That is not because the child has matured suddenly or rapidly; he has been maturing all the time. The preosseous tissue was not prepared for bony deposition, perhaps because the absence of some catalyzer, shall we say, prevented deposition of the mineral.

Now if you take breast-fed babies at 3 months and compare them with certain artificially fed babies at 3 months, the latter babies have an epiphyseal ossification pattern of 3 months on our standards of epiphyseal sheaves, and the breast-fed babies have an epiphyseal pattern of 2 months. These particular artificially fed babies get a considerable amount of vitamin D, and the breast-fed babies in the same city do not get that much vitamin D. The speed with which ossification centers begin to ossify seems related to the amount of vitamin D assimilated.

Now we come to the use of the aluminum gauge. Dr. Bakwin is right when he says the density gauge is a crutch; and it must not be allowed to overshadow the real test, namely a study of the texture itself not the density, which may be influenced by the processing.

Dr. Stuart asks whether X-ray films of joints might possibly differ in density because of the density of the skin or of subcutaneous tissues. That might be so if it were not that we are looking at the intra-articular structures, the *ligamenta alaria*, for example; the margins of the joint cavity. It is not the density; it is a morphological pattern which is to be distinguished.

This study of density may be made on both the child and the adult; we have had greater experience with the child than with the adult. Our records of the adult, which are now increasing quite vigorously, show that chronic fatigue from the pace of living is probably one of the greatest incentives to density of the subcutaneous tissues.

In reply to Dr. Palmer's question, I may say that all investigations go through three stages: first of study, then of service, and then of independent research. This investigation is just emerging from the study period to the period of service. Supposing we were going to make a survey for public health, what X-ray would I take? Well, it would depend on what I was wanting to investigate.

I should like very much, if it could be afforded, to take two X-rays,

if the study were of children. I should like to have both the region of the elbow, including the biceps, and the hand. That would show maturation; it would show density of the bone; it would show muscular size and density; it would show subcutaneous tissue density; it would show skin. I should, therefore, have a fairly satisfactory survey of the general condition of the child.

But suppose we were dealing with people at ages between 20 to 30. I should like still to take a picture of the hand, and I should like to take a picture of the elbow, because I would get everything above mentioned with the one exception of maturation which is now completed. For, after all, maturation is a problem that relates only to children. The other features relate to all phases of life. If, however, I were asked what X-rays I would take in order specifically to include maturation in young people in the upper teens, I would ask for one of the hip in order to get the iliac crest, because that is the only epiphysis except those of the vertebral column, which is available for X-ray and can be utilized at that particular period.

How far are these various points or features related to clinical health or clinical sickness? That we cannot say specifically. I have used allergy as an exploratory study, with the inevitable result, of course, that folk have been inclined to attach these specific appearances to allergy, but they are not specific in any way; they are characteristics of a disturbance. They are not specific features of a disease.

Dr. Harold C. Stuart: May I answer Dr. Palmer's question on public health? It is my opinion that X-ray films should be used by physicians more extensively in practice for a considerable period of time before they would be taken up in a public health program which would involve large expenditures. In the first place, we need to know more about what can be done in the way of securing adequate technique in general use. In the second place, we do not yet know about the significance of the findings which might be recognized to interpret them properly in a public health program. In the third place, the absence of findings could not be accepted as indicative of the absence of a need for attention to diet. For example, if we do not find specific evidence of scurvy by the X-ray, it is not indicative that the level of ascorbic acid in the blood is high. If we do not find evidence of rickets in the bones, it is not indicative that the child is having an optimum intake of vitamin D or calcium.

It seems to me that the use of the X-ray in a general health program at the present moment might lead to a great deal of misconception and harm. It might be used to advantage in certain surveys in a limited way, but I should like to urge rather that physicians in practice use the X-ray films that are available to them more intensively. In hospital conferences, one not infrequently sees an X-ray film demonstrated as showing nothing of interest, in which one can see features that may have real significance in respect to the development of the child. We have not learned to look for these evidences. I should like to urge, therefore, that the roentgenogram be taken up more definitely by physicians in connection with the growth and nutrition of children, but that its use for these purposes in connection with public health programs be delayed until we can say just what can be learned from it.

Dr. T. Wingate Todd: It has been asked whether there is some kind of relationship between the height and weight of our children and the maturation of our children. I hesitated, until I got that question, to say anything on the subject. From paper manikins it may be seen that two different boys from very different families show a very different relationship between their stature and their maturity; or, if you like, between their chronological age and their maturity. If you take a straight edge and run it along at any comparable part of successive manikins, you will find that although these are different boys, there is a definite rate of increment. Growth, in other words, in our children is not a matter of alternative spurts and slumps, but is a steady and predictable progress. These, of course, are well-circumstanced children.

If we would assort our growing children on the basis of maturation rather than on the basis of chronological age, we would get a small disparity or dispersion of stature particularly—I do not yet know how much in weight. Although I have not as yet subjected such data to statistical analysis, this conclusion has been reached from a study of individual growth patterns of more than one thousand of our children.

# DARK ADAPTATION AND THE DIAGNOSIS OF AVITAMINOSIS A

SELIG HECHT[1]

## I. VITAMIN A AND VISION

IT HAS long been known that certain visual disturbances such as nightblindness are associated with states of nutrition, and in recent years this association has become directed to the vitamin A in the diet. The reason for this correlation is that vitamin A is chemically related to visual purple, the light-sensitive material in the rods of the retina. Wald (1935) found that when the retina is illuminated and its visual purple bleached, one of the photoproducts is a carotenoid—retinine—which changes into vitamin A in a short time. Since the formation of visual purple in the retina depends on the presence of vitamin A (Fridericia and Holm, 1925; Tansley, 1931), it follows that vitamin A is a precursor as well as a product in the chemistry of the visual cycle.

Once the relation between vitamin A and the visual cycle became established, it seemed logical to use the properties of vision as a diagnostic sign for the vitamin A condition of the body (Edmund and Clemmesen, 1936; Jeans, Blanchard, and Zentmire, 1937; Jeghers, 1937; and others). Some of the work done in terms of this idea is perhaps a little more enthusiastic than critical; still, it shows that the idea is sound, and that under properly controlled conditions some visual properties may be used in diagnosing a vitamin A lack long before it becomes clinically evident.

In America dark adaptation has been the visual property used for diagnosis; and because our laboratory has studied the physiology of dark adaptation for many years, I have been invited to present the results of our work for the consideration of this Conference. The task assigned to me is two-fold. It is, first, to be didactic—that is, to describe what is known about the nature of

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dark adaptation as one aspect of the physiology of vision; and secondly, to illustrate our methods of measuring dark adaptation with a few examples of what may happen under changed bodily conditions involving vitamin A.

## II. RETINAL STRUCTURE

Though we speak easily about visual purple, rods, cones, retina and the like, most of us non-ophthalmologists are hazy about their precise meaning. The rough diagrams in Figures 1, 2, and 3 will help dispel this haze. Figure 1 is a horizontal section of the eye. Special emphasis is to be laid on the retinal depression opposite the cornea and lens: this is the *fovea centralis,* the region of sharpest vision. Though practically all our visual work is accomplished with it, the fovea occupies a very small portion indeed of the visual field.

Light enters the eye and falls on the retina, where it is absorbed by the sensitive structures. In these it produces photochemical changes which then initiate nerve impulses to be conveyed over a system of nerve and ganglion cells into the optic nerve and to the brain. Figure 2 is a section of the retina taken below the entrance of the optic nerve, and it shows that the light must pass through much nerve tissue to get to the sensitive elements. These receptor elements are of two kinds. First there are the rods, in whose terminal segments one finds visual purple. This photosensitive pigment is a conjugated protein — a carotenoid-protein — whose molecular weight is about 270,000 (Hecht and Pickels, 1938); it has been extracted and studied for many years (Kuehne, 1879; Garten, 1906; Hecht, 1924; Wald, 1935; Chase and Haig, 1938).

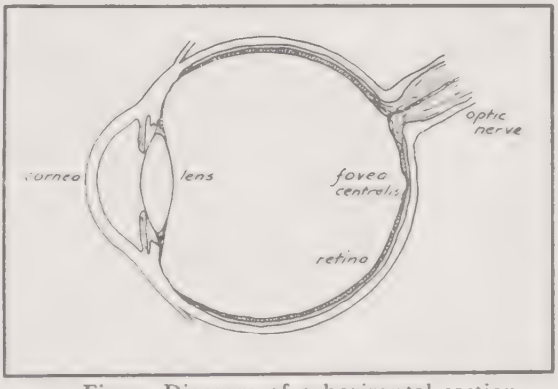


**Fig. 1.** Diagram of a horizontal section of the eye as a whole, showing the form and position of those structures which are necessary for an understanding of dark adaptation.

The rods are con-

cerned with vision at dim illumination, and such vision is always without color. Many rods are connected to one optic nerve fiber, so that rod-vision cannot be very sharp.

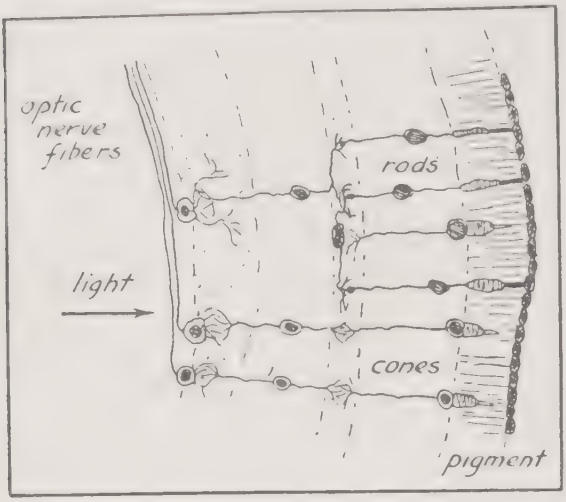


Fig. 2. A magnified section of the retina only, made below the entrance of the optic nerve. Note the rods and cones, and the optic nerve fibers. Note also that several rods connect with one nerve fiber; this is true of all the rods. Only the cones within the fovea have a one-to-one connection with optic nerve fibers, while outside the fovea several cones connect with one nerve fiber.

The other receptors are the cones, which are concerned with color vision at higher illuminations. Very recently Wald (1937) has been able to extract a photosensitive pigment from the cones, and we (Haig, Hecht, and Patek, 1938; Hecht and Mandelbaum, 1938) have found indirect evidence to show that this pigment is in all probability also a carotenoid-protein. It is called iodopsin or visual violet.

Figure 3 shows the visual field apparent on looking into the eye with an ophthalmoscope. It contains as prominent landmarks, the optic disc and the blood vessels, and to the right of the disc a spot which is the fovea. In this field, representing the surface of the retina, the distribution of rods and cones is not uniform as one might naively suppose, but is arranged in a definite pattern. The central region of the fovea corresponding to about 2 degrees of visual angle, is practically rod-free. In this center of vision there are no rods—only cones so tightly packed that there are about 150,000 cones per square millimeter. The number of cones quickly decreases towards the periphery. At the edge of the rod-free area the population is only 75,000 cones per sq. mm.; 10° farther out it is 5,000 cones per sq. mm. and remains this way.

On the other hand, the rods, after their appearance at the edge of the fovea, increase rapidly in number. At 2° from the

center there are already 60,000 per sq. mm. Their number reaches a maximum of 150,000 per sq. mm. at about 18° out, after which it again decreases until the edge of the retina.

Hand-in-hand with this anatomical distribution of rods and cones goes the function of the retina. Color vision, daylight vision, and good visual acuity are mediated by the cones; these functions are at their best in the fovea and decrease in excellence toward the visual periphery. On the other hand, vision at low illuminations is poor with the center of the eye, but improves as one uses the more peripheral parts of the retina.

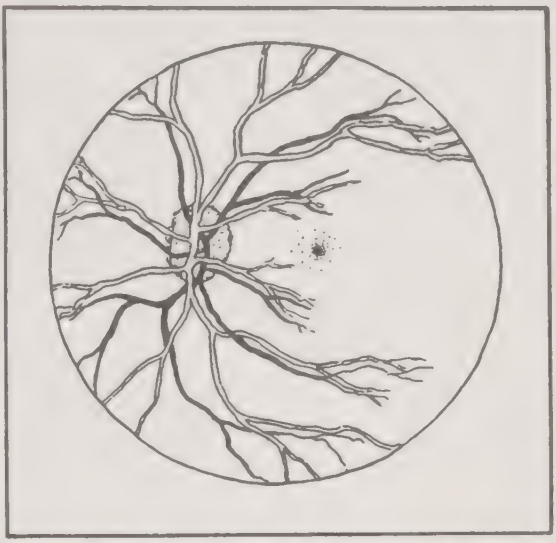

Fig. 3. The retina as it appears when examined with an ophthalmoscope. Note the optic disc, which is the entrance of the optic nerve fibers and the blood vessels. The central pigmented area is the fovea.

From this distribution of sensitive elements and visual behavior, it is apparent that in order to use the visual properties of the retina for diagnostic purposes one must have a clear notion of the nature of the function and of its location in the eye. This is particularly true of dark adaptation.

### III. DARK ADAPTATION

What is dark adaptation? In essence it is a familiar phenomenon. On coming from the brightly illuminated outdoors into a dimly lighted room, one can hardly see anything. Gradually, however, objects begin to take on shape, and in a few minutes one can even begin to see some detail. After half an hour, things appear so clearly that one is surprised at the initial visual obscurity. The process of achieving this good vision in the dark is called dark adaptation. It was first described in 1865 by Aubert and was first measured by Piper in 1903.

It is measured by determining the minimum amount of light which must illuminate a given surface in order to render it visible. What is found is that this threshold illumination is high on entering a dark room, and that it decreases in the dark. The extraordinary thing about this change in visual threshold is its range: it may easily cover a change from 100,000 units of light intensity at the beginning to 1 unit at the end of dark adaptation.

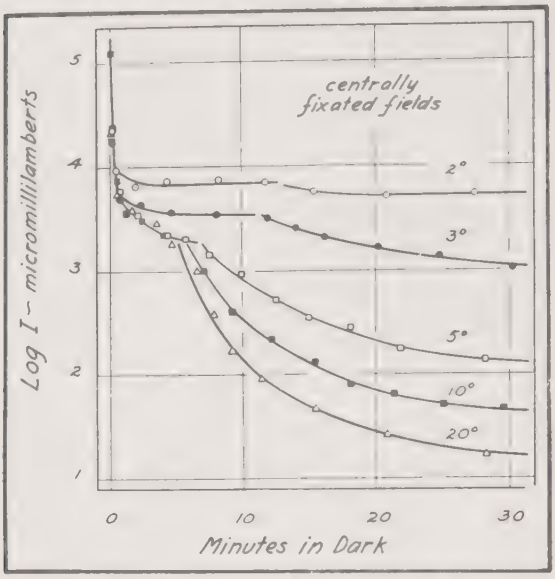


Fig. 4. The threshold during dark adaptation for centrally fixated areas whose diameters are shown on the data. The larger the field, the sooner does the secondary rod section appear, and the larger is the intensity range covered by it. The data are from Hecht, Haig, and Wald (1935).

Because dark adaptation is a threshold phenomenon, and vision at the threshold is colorless, the early workers supposed that dark adaptation is a function of rod vision alone. However, seventeen years ago I showed (Hecht, 1921) that the cones also undergo dark adaptation, and to nearly the same extent as the rods. Cone adaptation is much faster than rod adaptation, and this is the reason that the earlier investigators missed it in the course of their leisurely measurements.

In terms of the structure of the retina, it must be clear that if measurements of the threshold are made with a visual field which includes only the central rod-free area of the retina, the resulting data will record only cone dark adaptation. However, if a larger retinal area is used, or if the measurements are made excentrically, the resulting dark adaptation will record not only cone behavior but also rod behavior. This is illustrated by Figure 4 from the work of Hecht, Haig, and Wald (1935).

Before studying the data in Figure 4 it is necessary to say a

word about the units used in plotting them. Time on the abscissas is in minutes on a linear scale. Light intensity on the ordinates, however, is plotted on a power or logarithmic scale. There are several reasons for using a logarithmic scale, but the most important is the large range of intensities. The lowest ordinate (1) means 10 units; the next (2) means 100; the next (3) means 1,000 units, and so forth. A simple linear plot would have to be very large indeed in order to show any detail and would cramp some portions of the data and exaggerate others. Another virtue of a logarithmic plot is that the percentage error always occupies the same distance on the plot. Thus a 10 per cent error is always the same size whether applied to 1 unit or to 1,000 units. Now to return to the data.

In Figure 4 the top curve is the course of dark adaptation measured with a centrally-fixated area 2° in diameter. The drop in threshold is very rapid, and is over in about 3 minutes: it is purely cone adaptation. However, since there are a few rods on the edges of this region, their slow adaptation becomes visible as a slight secondary drop in threshold appearing after 15 minutes in the dark. The second curve records adaptation with a 3° field centrally fixated. Such an area contains more cones than a 2° field, but it contains many more rods, and this is made evident by the increased size of the secondary drop in threshold. It is apparent that as the retinal area increases, the primary cone section of the data also increases in range, but the increase is small in comparison to the increased range which the secondary rod section shows.

The moral of all this is that in order to make measurements of dark adaptation which have meaning, it is necessary to specify the size of the area used for measurement. However this is not enough, for there are other factors which influence the appearance of the data. Figure 5 shows what happens when a 2° area is used but its location is altered. Placed centrally (at 0° from the visual axis) it measures practically only cone adaptation. Placed peripherally the range of rod adaptation increases strikingly as one goes outward. In fact, as comparison between Figures 4 and 5 indicates, a 2° field placed 21½° excentrically shows as much

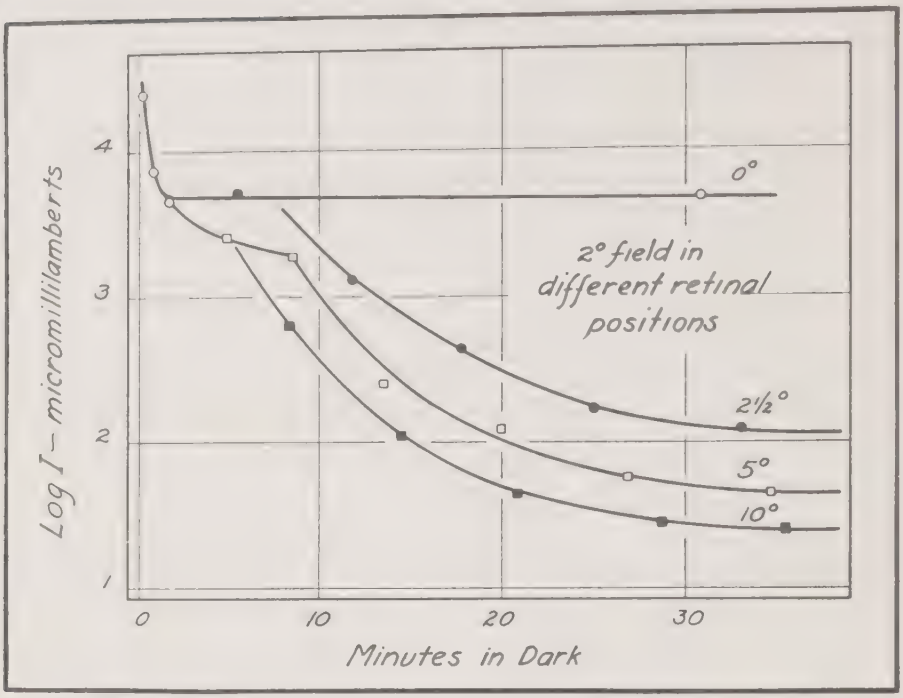


Fig. 5. Dark adaptation as measured with a 2° field placed at the different distances from the center recorded on the data. Compare this with Figure 4 for centrally-fixated fields of different size. The measurements are from Hecht, Haig, and Wald (1935). Note that the farther in the periphery the measurements are made, the sooner does the rod section appear, and the lower is the final threshold.

adaptation range as a 5° field placed centrally; and a 2° field viewed 5° excentrically corresponds to a 10° central field, etc. It follows that not only the size but the exact location of a retinal area must be specified in measurements of dark adaptation.

A third factor which influences the course of dark adaptation is the color of the light used for the measurements. The rods and cones have different sensibilities in the spectrum (Hecht, 1937). In the extreme red the two have very nearly the same threshold, but in the blue the cones need about 1,000 times as much light to be stimulated as do the rods. For intervening portions of the spectrum the relative sensibilities are intermediate between these two extremes. Figure 6 shows the measurements of Kohlrausch (1931) for the dark adaptation of the same retinal spot under the same conditions, but with lights of different colors. With extreme red light, because the rods and cones have the

same threshold, only the more rapid cone adaptation is evident. But with the other colors both the primary cone adaptation and the secondary rod adaptation become apparent. And as the light goes toward the blue the range of the rod adaptation becomes steadily greater, reaching its maximum with blue light.

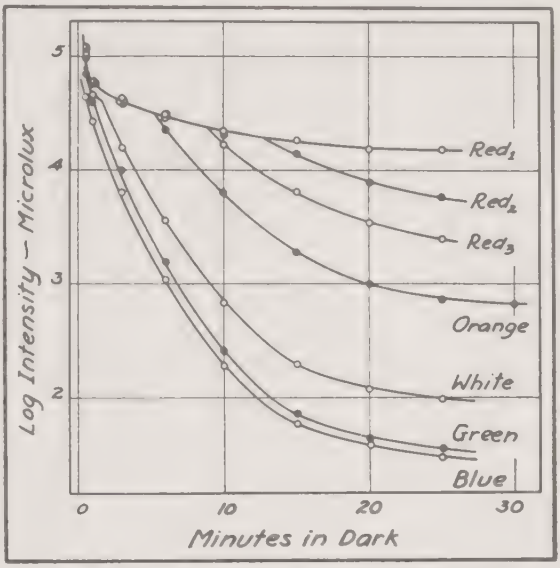


Fig. 6. The dark adaptation of an area 1° in diameter, situated 5° above the fovea (Kohlrausch, 1931). $Red_1$ is the only color which limits the measurements to the cones. The other two reds let through more orange light, and show up rod adaptation. With this intensity of preadaptation, cone adaptation is barely evident with blue light. *Cf.* Figure 7.

Dark adaptation is recorded by a series of measurements of the visual threshold. It is well known that the light intensity necessary for threshold vision varies inversely with the exposure of the eye to the light. A short flash of light must use a brighter light in order to be visible than does a long flash. The relation between time of exposure and intensity of light for a threshold effect is different for the cones than for the rods. Therefore not only the position of the dark adaptation curve on the intensity axis but the relative extent of cone and rod adaptation will be determined by the duration of the test light used for the measurement of the threshold.

So far we have considered only the measurements of dark adaptation itself. However, dark adaptation presupposes a previous light adaptation; and the conditions of this light adaptation influence profoundly the course of the subsequent dark adaptation. Figure 7 shows how the intensity of the preadapting light affects the dark adaptation which follows it (Hecht, Haig, and Chase, 1937). With a very high light preadaptation, cone adap-

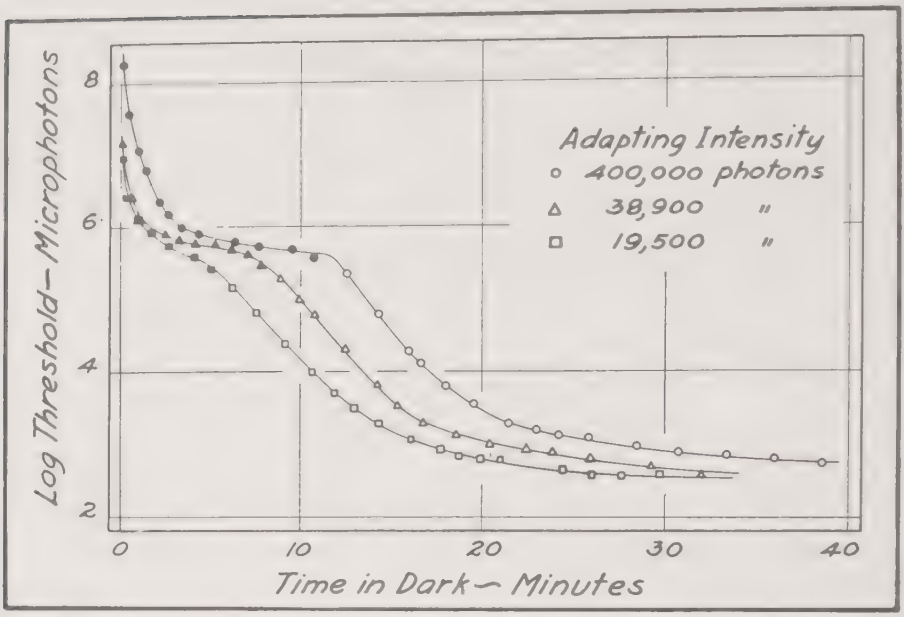


Fig. 7. The dark adaptation of a 5° field situated 30° nasally (Hecht, Haig, and Chase, 1937). Though measured with extreme violet light, the course of adaptation shows two distinct sections, due to the high light adaptation preceding the measurements. Note that the secondary rod adaptation appears later the higher the intensity of preadaptation.

tation is rapid, but rod adaptation is delayed for about 12 minutes, and lasts for over 40 minutes. As the preadaptation brightness decreases, the extent of cone adaptation also decreases, and rod adaptation appears sooner, until one may find an intensity of preadaptation which is followed only by rod adaptation. Note that even the shape of the rod dark adaptation curve changes with the intensity of the preadapting light.

Not only does the intensity of the preadaptation influence dark adaptation, but the duration of the preadaptation controls it as well. Figure 8 shows the measurements of Müller (1931). With a 1 minute light adaptation to this particular brightness the subsequent dark adaptation is pure rod function. As the time of light adaptation increases one gets more and more cone adaptation, and the rod adaptation comes later and later.

*From these considerations it follows that there are at least six specifications which must be made in recording dark adaptation adequately.* These are the intensity and duration of the preadaptation light, and the area, the retinal location, the color, and

the duration of the measuring light. It is to be regretted that most of the clinical measurements about the relation of dark adaptation to vitamin A have been made in apparent ignorance of the information so far presented.

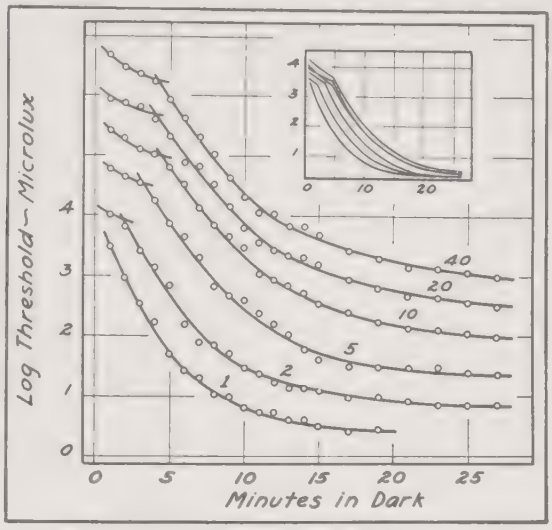


Fig. 8. Dark adaptation of the eye after preadaptation for 1, 2, 5, 10, 20, and 40 minutes to 3,000 lux (Müller, 1931). The time axis is the same for all the curves. The ordinates apply only to the lowest curve; for convenience, each series is displaced 0.5 log unit above the other. The inset brings the data together, and shows that the longer the preadaptation, the more evident is the primary cone dark adaptation, and the more delayed is the secondary rod adaptation.

## IV. NORMAL VARIATION

The didactic section of my task has now been accomplished. There remains to be presented our more recent researches which bear directly on the problem at hand, namely the relation between vitamin A and dark adaptation.

As the result of many requests for assistance and advice, Shlaer and I (Hecht and Shlaer, 1938) have designed an adaptometer which can be used in physiological and clinical investigations. The instrument was built in the spring of 1937, and has been in constant use in our laboratory since; moreover, several duplicates have been made and are in use in other laboratories.[2] It not only incorporates all the six specifications which I have outlined, but is so arranged that the specifications can be varied in order that any aspect of dark adaptation may be studied under different but controlled conditions. Essentially it is a device for adapting the eye to white light for a given time, and for measuring the subsequent adaptation of a fixed area with violet light. So as not to interrupt the sequence of

[2] These instruments, as well as our own original model, were constructed by Mr. O. C. Rudolph, 55 Van Dam Street, New York City.

ideas, the description of the adaptometer and the method of its use are placed at the end of this paper in Secions VIII, IX, and X; and we may now proceed on the assumption that we have such a flexible instrument at our disposal.

Since the course of dark adaptation depends on so many conditions, our first task was to ascertain the best conditions for routine determinations of dark adaptation. Because the precise effects of nutritional states and pathological conditions on the course of dark adaptation are not known, it seemed advisable to choose such a light adaptation that both cone adaptation and rod adaptation will be separately and adequately represented in the results. This is achieved when the brightness of the light-adapting field is 1,500 millilamberts.

For dark adaptation we selected a test field 3° in diameter as a compromise between a smaller field which would report the behavior of a reasonably uniform portion of the retina, and a larger field which can be more easily seen by untrained observers. The test field and the light-adapting field are viewed 7° nasally, using the right eye. At 7° nasally, the retinal populations of rods and cones are more nearly equal than in the center or farther in the periphery. In addition, the luminous fixation point viewed by the central fovea is sufficiently removed from the measuring region of the retina so that it does not interfere with the observations. The test field is viewed with light from the extreme violet of the spectrum. Two things result from this choice, as may be seen from Figure 9 which records the data of a typical inexperienced individual making the measurements for the first time. First, the rod section of the dark adaptation curve is maximal in range, and secondly, there is a sharp and easily noticeable color difference between those threshold measurements which are made with the cones, and those made with the rods. Even the most inexperienced observer will record the fact that at the threshold the lights corresponding to the first section in Figure 9 are all colored blue or violet, while those corresponding to the second part of the curve in Figure 9 show no color at the threshold.

The measuring light is exposed in flashes of one-fifth of a

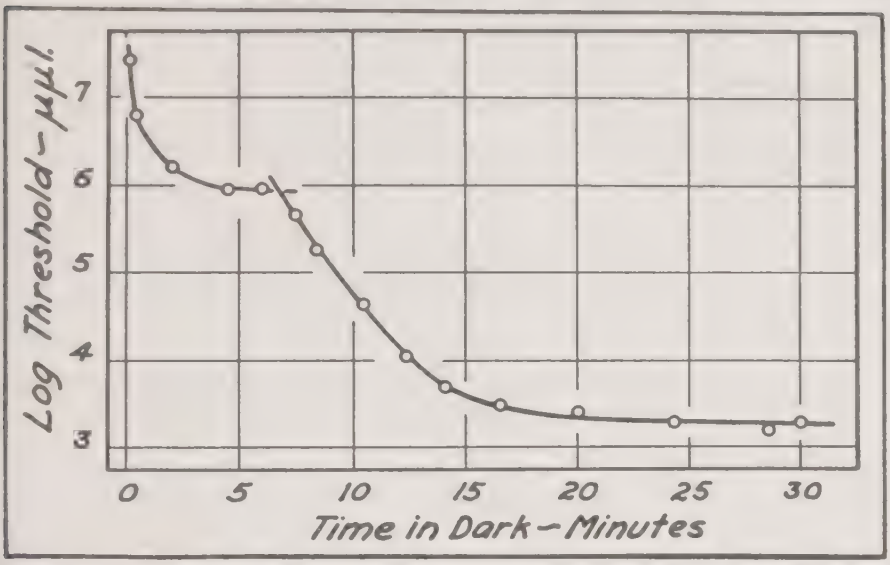


Fig. 9. The course of human dark adaptation following light adaptation to a brightness of 1,500 millilamberts. The points are single measurements and record the threshold of a retinal field 3° in diameter and situated 7° nasally. The measuring light is from the violet end of the spectrum below 460mμ. The first section of the data records cone function, while the second section records rod function. This is evidenced, among other things, by the fact that even at the threshold for the first five points the light appears definitely blue, whereas for the remaining points the threshold is without color. The ordinates are in micromicrolamberts (*μμl*).

second. This is long enough to produce a good perception of the test field during dark adaptation and short enough to be near the physiological limits of the retinal action time.

Once these conditions of measurement were established, Dr. Joseph Mandelbaum and I made routine measurements with 110 individuals of both sexes between 15 and 65 years old. Most of our subjects were university people, either students or faculty. This was convenient; but it was also useful because such people are usually well-nourished and their diet is most likely well-balanced.

There are three aspects of the dark adaptation curve which vary from person to person: the final cone threshold, the final rod threshold, and the time of the cone-rod transition. Figure 10 shows the distribution of these characteristics in the group as a whole. Age affects neither the final rod threshold, nor the time of cone-rod transition; but it does affect the final cone threshold

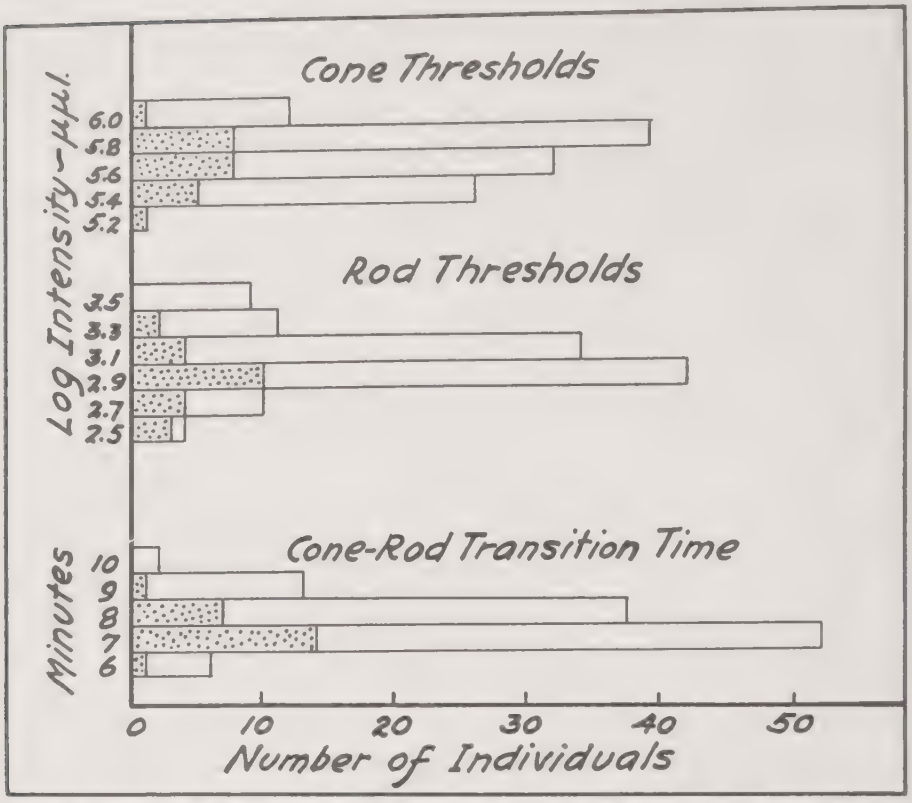


Fig. 10. The distribution of cone thresholds, rod thresholds, and cone-rod transition times in a population of 110 well-nourished individuals of university connections. Neither age nor sex makes any significant difference in the distribution of rod thresholds, and cone-rod transition points; cone thresholds, however, move perceptibly with age, going from a mean of 5.56 log units for the age group of 15-21 years to a mean of 5.83 log units for the age group of 40-65 years. The stippled areas indicate females; the clear areas males.

which becomes higher the older the group. About 80 per cent of the group show a cone-rod transition between 7 and 8 minutes in the dark, and a rod threshold which falls within 0.20 log unit to either side of the mean threshold whose logarithm is 3.02. The cone threshold of the group covers a wider range due to the age spread. Figure 11 shows four curves of dark adaptation. The upper and lower curves are the extremes of the group, while the middle two cover the range within which about 80 per cent of the group falls. To judge by these measurements, most people will fall within the middle two curves. A value of 0.40 log units corresponds to an intensity variation by a factor of 2.5.

Six of our subjects made repeated runs, five or six times over

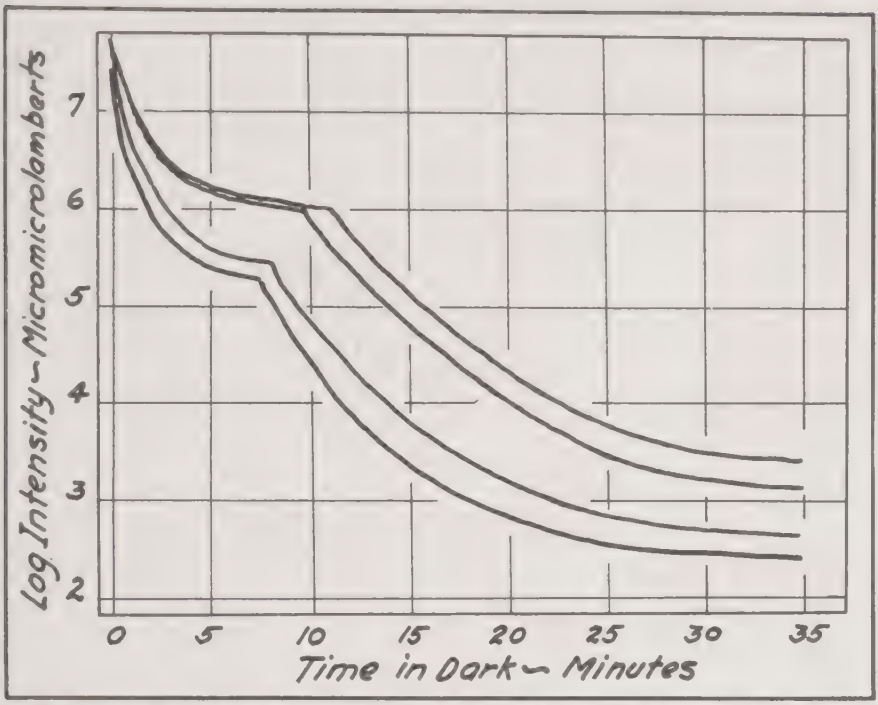


Fig. 11. Four actual curves of dark adaptation. The upper and lower curves are the extreme variates found in our survey, while the two middle ones include between them 80 per cent of the group.

a period of 4 weeks. The threshold variation encountered in this way for each person corresponds well with what we have repeatedly found in measurements of vision, namely that a given individual will show a day-to-day variation of about 0.3 log units, that is by a factor of 2 in the actual intensity. This means that if the threshold value today is 1, then next week it may be 2, and it will continue to lie between these two values. Considering that the total threshold change during dark adaptation is about 4 log units, or 10,000 to 1, this is a comparatively small variation.

## V. AVITAMINOSIS

After having established the normal range to be found in a reasonable sampling of individuals, we (Hecht and Mandelbaum, 1938) made the experiment of decreasing the vitamin A intake of the body and following the dark adaptation. We were interested in finding out which of the characteristics of the curve is affected, and in particular whether the cone threshold changes

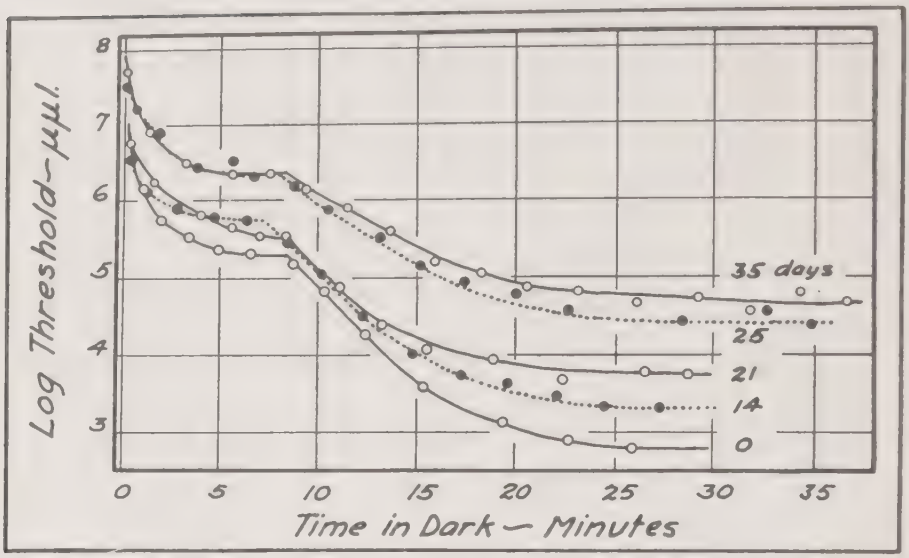


Fig. 12. Dark adaptation curves made at various times during a vitamin-A-free diet. Each point is a single observation. Data and curves are from Hecht and Mandelbaum (1938).

as well as the rod threshold. We measured four normal young men, first on their regular and adequate diet, and then on a diet containing only about 150 units of vitamin A per day. Jeghers (1938) has already made measurements of dark adaptation on one person subjected to vitamin A deficient diet, but due to inadequate apparatus and procedure his data do not even separate rod and cone adaptation and thus do not clearly tell us what happened as a result of the avitaminosis.

Our measurements were made as already described with the new adaptometer. Figure 12 gives five curves of dark adaptation taken with one subject during different stages of the deficient diet. The results show clearly that cone function is affected by vitamin A changes, just as rod function is affected by them. However, rod vision is affected more than cone vision in that the rod threshold rises faster than the cone threshold. The cone-rod transition point remains the same during all the vicissitudes of the diet, and this is true for all four subjects.

The parallelism in behavior of rod and cone functions is best shown in Figure 13, where the data for two subjects are drawn. Those for J. M. are about average, while L. W. represents the

extreme effect of the four subjects. Each point is the final threshold secured either from the cone or the rod sections of such curves as in Figure 12. All subjects had been studied for several weeks before beginning the diet, and the thresholds from the last two runs made on the normal diet are shown above the diagonally shaded area in Figure 13. The clear area represents the duration of the deficient diet. The similarity in behavior of cones and rods in dark adaptation is obvious.

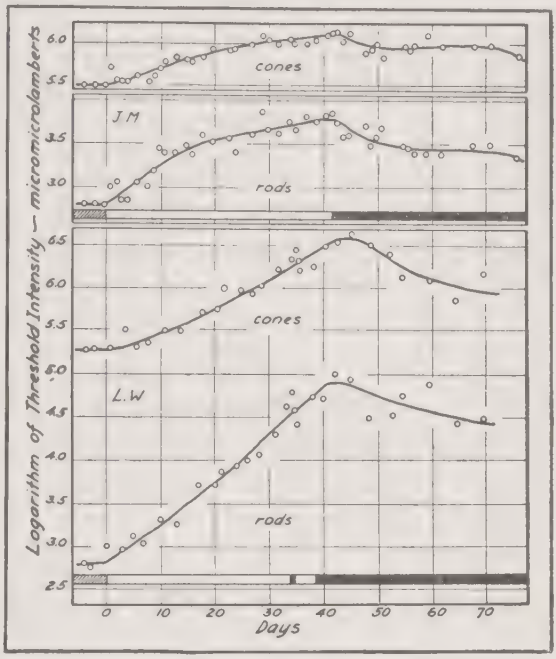


Fig. 13. The final rod and cone thresholds of two subjects on a vitamin A controlled diet. The diagonally shaded part represents a normal diet; the clear part is a practically vitamin-free diet; and the black part represents a normal diet plus 50,000 units of vitamin A daily. Note the points above the narrow black area for L. W. Both for rods and cones, this single administration of a large dose of vitamin A (100,000 units) seemed to cause a drop in threshold during the same day. In view of the prolonged time subsequently required for the threshold to come down to normal on a supplemented diet, we have not taken these points too seriously. Data and curves are from Hecht and Mandelbaum (1938).

For our present purpose, the important thing which Figure 13 brings out is that as measured with dark adaptation the presence of an avitaminosis becomes evident after the very first day of the absence of vitamin A in the diet. Naturally, because of the normal variation found, no reliance can be placed on such a measurement. But note that after a week of vitamin A lack the rod threshold in Figure 13 has risen well beyond the normal range, and a diagnosis can be made with reasonable certainty.

The black area in Figure 13 means the return to a normal diet supplemented by 50,000 units of vitamin A per day. L. W. received 100,000 units in one day, but became ill for a few days

following, during which he ate almost nothing and only resumed a supplemented normal diet later as indicated. The other two subjects merely returned to a normal diet without supplementary vitamin A. As Figure 13 shows, there has been no spectacular return of visual function to normal on the resumption of regular and even excessive vitamin A consumption such as has been reported previously (Jeghers, 1938; Edmund and Clemmesen, 1936). There is a slightly rapid drop in threshold at first, but this gives way to a gradual decrease in threshold, and it may take longer to achieve complete recovery of function than it took to lose it.

### VI. CIRRHOSIS OF THE LIVER

The flow of vitamin A to the retina may be interrupted not only by its elimination from the diet, but by the breakdown of any one of the organs concerned in its transportation within the body. For example, since the liver is the chief depot for storage of vitamin A (Moore, 1931), it is probable that disease of the liver would disturb the metabolism and storage of this vitamin. Indeed, nightblindness and keratomalacia have been reported in liver disease (Hori, 1895; Jeghers, 1937). This was pointed out to us by Dr. Arthur J. Patek, Jr., whose own observations on persons with alcoholic cirrhosis of the liver showed that some of them possess evidences of nutritional deficiency such as lesions of the skin and cornea which suggest the specific lack of vitamin A.

With Dr. Patek's help we (Haig, Hecht, and Patek, 1938) studied several of his patients using our newly designed apparatus even before we had decided on a standard procedure and had completed our normal survey. Hence the white preadapting brightness was 1,600 millilamberts and was viewed by the subject for 4 minutes; moreover the test field was 4.5° in diameter, and was located 8.5° nasally in the right. These specifications are near enough those we finally adopted, as already described, that no further attention need be paid to them.

Of the 14 persons with alcoholic liver cirrhosis tested, 13 showed plain evidence of disturbances in dark adaptation. Two

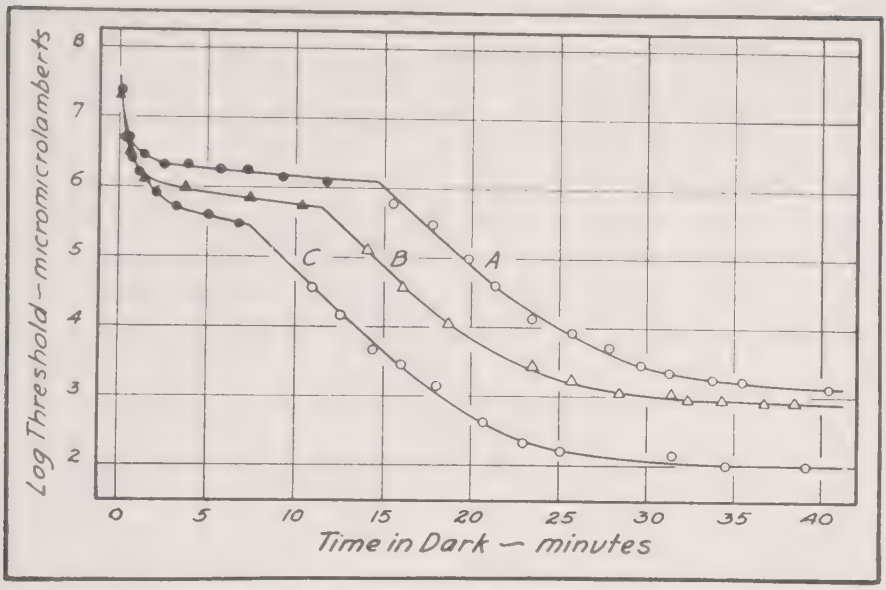


Fig. 14. The course of dark adaptation of a person with alcoholic cirrhosis of the liver determined at different times during vitamin A therapy. The points are single measurements and record the visual threshold to violet light during a stay in the dark following 4 minutes light adaptation to 1,600 millilamberts. Those measurements which even at the threshold appear blue or violet to the subject are represented as solid symbols; they all fall on the primary cone portion of the curve. Those measurements which are reported as colorless at the threshold are shown by unfilled symbols, and represent the secondary rod portion of the adaptation. Curve *A* was obtained when the subject was on an ordinary diet. Curves *B* and *C* were obtained after 105 and 127 days respectively of vitamin A therapy. Note that both the cone and rod thresholds vary and that the cone-rod transition point varies in different stages of the treatment. Curves are from Haig, Hecht, and Patek (1938).

of these patients were fed large daily doses of vitamin A.[3] After 19 daily doses of 40,000 international units, the dark adaptation of one patient became normal. The other patient, whose liver cirrhosis was more extensive, responded more slowly and exhibited normal dark adaptation only after a ten-fold increase in the vitamin dosage. The measurements made on the latter patient will be considered in detail.

Figure 14 shows graphically three sets of these measurements made at different times during vitamin A therapy. Curve *C* is after 127 days of therapy and corresponds to that usually obtained

[3] We used oleum percomorphum, carotene, and a vitamin A concentrate. The vitamin A concentrate (free of vitamin D) was supplied to us by the Vitex Laboratories, Harrison, N. J., through the kindness of Professor T. F. Zucker, Columbia University.

with normal people. It records the way in which the light intensity threshold changes in the dark, and shows the usual rapid primary cone adaptation followed by the slow secondary rod adaptation. The transition between the two is sharp and occurs after about 7 minutes in the dark. The data marked *A* were obtained when the subject had been on an ordinary diet for three weeks; those marked *B* were obtained after 105 days of vitamin A therapy.

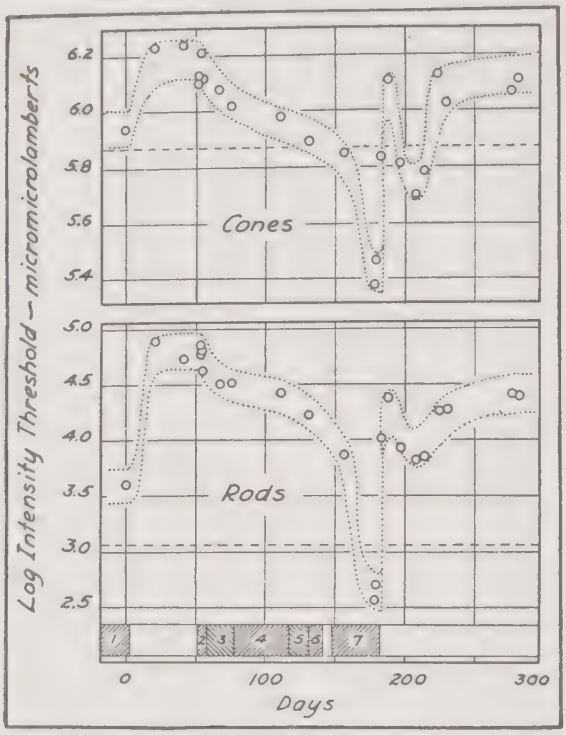


Fig. 15. Relation of cone and rod thresholds during dark adaptation to the sequence of vitamin A therapy. Daily vitamin A dosages in international units are as follows: (1) 40,000 units in oleum percomorphum, (2) 75,000 units in a D-free concentrate, (3) 60,000 units of carotene, (4) 60,000 units in oleum percomorphum, (5) 250,000 units in a vitamin D-free concentrate, (6) and (7) 500,000 units of the same concentrate. The clear spaces represent no vitamin A administration. Curves are from Haig, Hecht, and Patek (1938).

There are two points of significance in these data. First, the time of appearance of the cone-rod transition is much longer for curves *A* and *B* than for normal. During treatment this transition point moved from its initial value of 15 minutes until at the end of the treatment it occurred at 7 minutes, much as with the normal eye. Secondly, the *A* and *B* data show thresholds much above the normal, and this applies both to the cone section and to the rod section of the curves. During treatment the cone threshold was lowered by a factor of 5, and the rod threshold by a factor of 150—a ratio of 1 to 30.

The precise way in which the rod and cone thresholds varied in this subject is shown in Figure 15. The points record the posi-

tions of the cone threshold after 6 minutes in the dark, and the positions of the rod threshold after 20 minutes, and both positions are plotted against days of therapy. Since the rod threshold variation is about 30 times that of the cones, the thresholds of the latter have been plotted on a proportionately larger scale. The median threshold of 15 normal individuals is represented by a dashed line. Through the measured points there has been drawn a band 0.3 log unit wide for the rods, and 0.15 log unit wide for the cones. The width of the band represents the extent of the extreme day-to-day variation that is found with normal people, and also with untrained patients of this type. This does not represent variations in the apparatus or in procedure, but in function and physiological condition. The therapeutic treatment is indicated at the bottom of the figure. A clear space means a normal diet with no added vitamin A, while a filled-in space represents some form of added vitamin A as explained in the legend.

When the subject was first tested, that is at 0 days in Figure 15, he had been receiving 40,000 units of vitamin A daily for 58 days. At that time his cone threshold was only slightly higher than median normal and his rod threshold 0.55 log units above median normal. The rod data thus indicated a moderate degree of vitamin A deficiency. The treatment was discontinued, and 21 days later he was tested again. The thresholds of both functions had risen considerably. They fluctuated about this high level until the resumption of treatment, which took place on the 53rd day and continued with increasing daily dosage as indicated in the figure. Improvement was steady but slow until the dosage was increased to 250,000 and then to 500,000 units, when the threshold dropped precipitously to a point well below median normal. On discontinuance of treatment the thresholds rose rapidly, decreased again for about 20 days, then again rose to a high level.

## VII. DARK ADAPTATION AND DIAGNOSIS

As a result of our studies we are convinced that measurements of dark adaptation, when made under properly specified conditions, can be used as an aid in the diagnosis of the vitamin A con-

dition of the body. Normal dark adaptation means that the vitamin A content of the body is normal.

What normal is, still remains to be defined precisely. At present we may take it to mean the dark adaptation and vitamin A content of ordinarily well-nourished, healthy people. It may be that our common, normal dark adaptation can be improved by supplementary vitamin A feeding. Haig and I have not been successful in this regard, but our experiments did not last long enough. If it were successful, it still remains to be shown that it is desirable. For the present, therefore, normal had better be accepted in its ordinary usage.

When the dark adaptation of an individual is found to be abnormal, that means that his retina is not adequately supplied with vitamin A, unless he possesses an inherited hemeralopia (Hecht, 1937) or his cone function is congenitally deficient (Hecht, Shlaer, Smith, Haig, and Peskin, 1938). When such genetic factors have been excluded, it is then necessary to inquire into the basis for the retinal vitamin A lack. It may be diet, in which case the treatment is simple. It may, however, be some other link in the chain from diet to retina, such as the liver. Other diagnostic signs must then be looked for.

It is possible that further study will show characteristic disturbances of adaptation resulting from various diseases. Note that simple dietary avitaminosis does not change the time of the cone-rod transition point (Fig. 12), whereas cirrhosis of the liver delays its appearance to almost twice its duration (Fig. 14). This may be purely a matter of short-period avitaminosis versus prolonged deprivation. Only additional investigations can tell us, and the important thing is to make these investigations with adequate apparatus and control.

### VIII. CONSTRUCTION OF THE ADAPTOMETER

The apparatus with which these measurements were made consists of two parts—one is for adapting the eye to light, and the other for measuring its threshold during the subsequent dark period. For convenience in presentation we may consider first the light adapting arrangements, and then the measuring ar-

rangements. They are shown separately in top view in Figure 16; actually they are together in the adaptometer. The eye remains in a fixed position, and the change from light adaptation to dark adaptation is accomplished in a fraction of a second by the simple shift of a lever.

The optical system for light adaptation is shown in the upper part of Figure 16. The source is a circular ground glass window $G$ in a lamp housing which contains a 40-watt Mazda frosted lamp $M$ running on a fixed amount of current. The lenses $L_1$ and $L_3$ are arranged to produce an image of the source $G$ in the plane of the exit pupil $EP$. The result is that when the eye is placed at the pupil, the lens $L_3$ appears uniformly illuminated and covers an area whose diameter is about 35° visual angle. Between the source $G$ and the lens $L_1$ there is place for two filters $N$ and $C$ so that both the intensity and the color of the adapting light may be varied with neutral and colored filters. At present we keep a ¼ neutral filter here to bring the intensity to 1,500 millilamberts. About 5 cm. behind the lens $L_3$ there is a clear glass plate $P$ in the center of which is scratched a mark $X$ to serve as a fixation point. This plate can be slid horizontally by means of a convenient lever so that the light-adapting field may fall on any desired part of the retina, comparable to the part later to be tested for its dark adaptation by the measuring light.

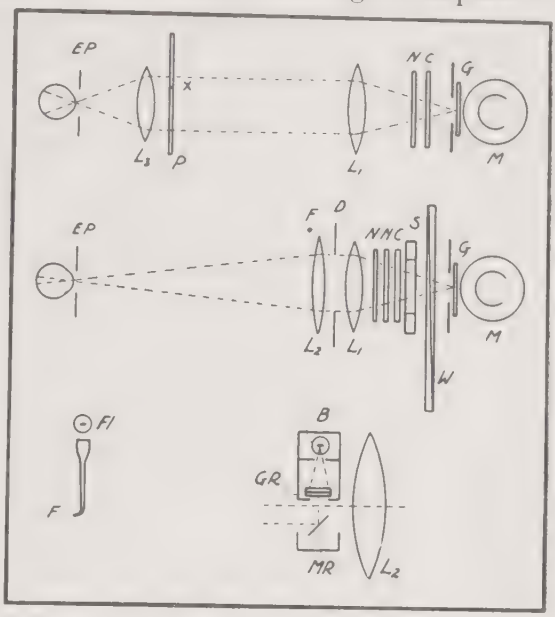


**Fig. 16. Diagrammatic representation of the optical parts of the apparatus. The upper portion of the figure is the optical system for light adaptation, and below it is the system for measuring dark adaptation. At the bottom to the left is the arrangement for the luminous fixation point, while to the right is the standard for calibration. The different parts are explained in the text.**

The optical arrangements for threshold testing during dark adaptation are shown in the middle of Figure 16. The lamp *M*, the ground glass source *G*, and the lens $L_1$ are the same as in the previous system. However, the lens $L_3$ and the glass plate *P* have been replaced by the lens $L_2$ which in combination with $L_1$ also images the source in the plane of the exit pupil, *EP*, so that the eye sees the lens $L_2$ as a uniformly illuminated circular field.

The exit pupil *EP* is an opening in a proper eyepiece into which may be put a lens for correcting the vision of the observer; this is necessary only under special conditions. If it is desired to keep the observer's pupil constant, this may be done by making the exit pupil small. This is necessary only for the most precise physiological work, because the change of the pupil from the light adapted condition to dark adapted condition is so small and happens so quickly that it hardly enters into the measurements.

The mount for the lens $L_2$ carries a groove for holding the metal diaphragm *D* which controls the field size. Just above the lens $L_2$ on the same mount is a rack carrying a luminous fixation point *F*, which may be placed in any position corresponding to that of the fixation mark *X* on the plate *P* used during light adaptation.

The fixation point assembly is shown separately in the lower left of Figure 16. It consists of a small lamp housing containing a flashlight-lamp *Fl*, the light from which is led down to the level of the center of the lens $L_2$ through a thin silvered and blackened glass rod, whose final open point *F* is thus small and brightly luminous. The brightness of the fixation point can be varied with either of two rheostats in series with the lamp. One of these may be operated from the observer's end, and the other from the recorder's. The entire fixation point assembly may be moved along its graduated rack and set to any position within 20° from the center to either side in the horizontal meridian.

Between the lens $L_1$ and the source *G* there is now an intensity control assembly consisting of a neutral tint gelatin-between-glass wedge, *W*, with its compensator, a shutter *S*, and a rack for two neutral filters, *NN*, and one color filter *C*. The wedge *W* is moved by a rack and pinion, and may be driven by either one of

two knobs: one at the front of the instrument for the observer, and the other at the rear for the recorder. The wedge covers an intensity range of 1 to 1,000; it is 15 cm. long, and its position may be read on a millimeter scale attached to it. By the addition of neutral filters at *NN*, the intensity of the illumination may thus be continuously varied to any required extent.

Even though it has these two optical systems, the adaptometer is a unit, and is properly housed in a blackened metal case, which contains screens and diaphragms wherever necessary. Some idea of the actual instrument may be gained from the photographs in Figure 17, which show it arranged for light adaptation and for dark adaptation. The instrument is used in a dark room, and is set in the wall of a cubicle which is open at the back, and in which the observer sits. This arrangement is shown in Figure 18, and is for the purpose of shielding the observer from stray light emitted by the ground glass *G*, or from the small lamp used for illuminating the scale of the wedge.

## IX. CALIBRATIONS

We use five neutral filters transmitting respectively 1/4, 1/10, 1/100, 1/1,000, and 1/10,000. These are calibrated with a Martens polarization photometer according to the method of Hecht, Shlaer, and Verrijp (1933). We also use two color filters, Wratten 88 which transmits only the extreme red of the spectrum beyond 680 $m\mu$, and Corning 511 which transmits only the extreme violet of the spectrum below 460 $m\mu$. These are calibrated for total transmission against white light with the Martens photometer. The wedge and its balancer are calibrated with white light and with each of the two color filters; similarly the neutral filters are calibrated not only for white light but for the two color filters as well.

With the wedge in place, it is necessary to determine the absolute value of the brightness visible to the eye at some one position of the wedge. This is accomplished by means of a calibrated standard specially arranged for this purpose, and shown in the lower right of Figure 16. A small battery lamp *B* running on a controlled current illuminates three layers of ground glass *GR*.

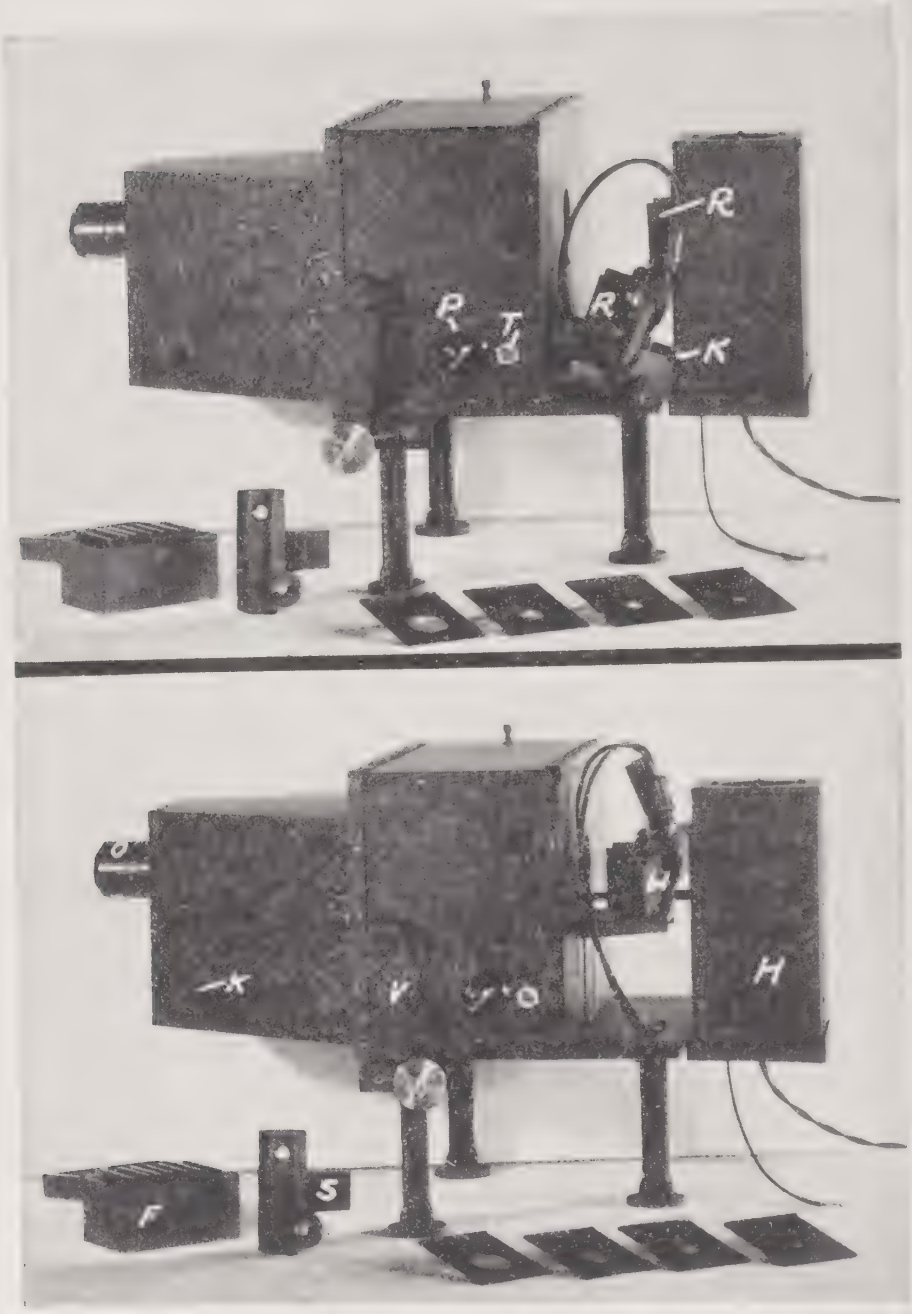


Fig. 17. The assembled adaptometer. In the upper picture it is arranged for light adaptation; in the lower for dark adaptation. The change from the one optical system to the other is made by shifting the lever *T*. Note the different position of the wedge-shutter-filter-rack assembly in the two conditions. The wedge is marked *W*, and the two filter racks *R*. The wedge is moved by either of two knobs *K*. The lamp housing is *H;* the switch is *T*. The rheostat knob for the fixation point is *P;* a similar rheostat and knob is on the side of the instrument not visible in the figure, and enables the subject himself to control the brightness of the fixation point. The ocular is *O;* the box containing the neutral and color filters is *F;* and the standard lamp and photometric device for calibrating the absolute brightness is *S*.

This is viewed through the circular opening by means of the mirror *MR* (a thin silvered cover slip). The current through the lamp is so regulated that the apparent brightness is 10 millilamberts as measured with a Macbeth illuminometer. The whole standard is properly encased and can be suspended in front of lens $L_2$ in the apparatus. Thus the eye at the exit pupil *EP* sees a circular photometric field, the left half of which is formed by the light from the lens $L_2$ and the right half of which is the mirror reflecting the light from the ground glass. By moving the wedge *W*, the position is then found at which the two are equal, and this sets a given position of the wedge as yielding an apparent brightness of 10 millilamberts. From this value and the calibrations of the wedge and of the filters one constructs a table showing the value of the brightness at any position of the wedge in conjunction with the necessary filters so as to give a continuously variable intensity for threshold measurements over a range of 1 to $10^{10}$ units.

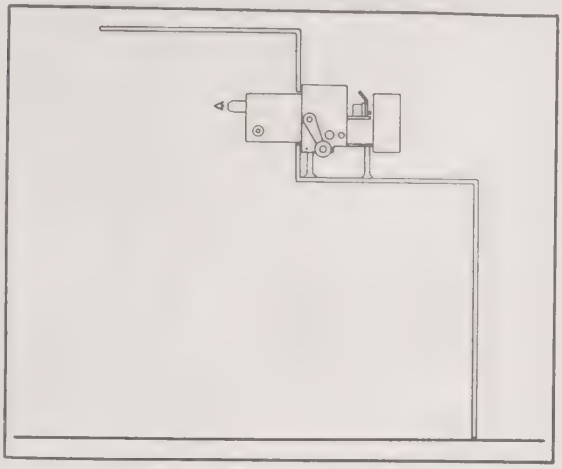

Fig. 18. The arrangement of the adaptometer in actual use. Its front end is inserted in the wall of a cubicle open at the back in which the subject sits comfortably. The subject's chin is in a chin rest so that his eye always comes to the correct position in front of the eyepiece as indicated.§

Most measurements of dark adaptation made with reasonably sized test areas reach a final threshold of about one millionth of a millilambert, which is nearly the same as one millionth of a foot candle. Since the threshold intensities are to be plotted in logarithms, which would have to be negative for these intensities, we have found it convenient to use a unit which is much smaller

§ Figures 4, 5, and 7 are reproduced from the *Journal of General Physiology;* Figures 6 and 8 from *Physiological Reviews;* Figures 9, 16, 17, and 18 from the *Journal of the Optical Society of America;* and Figures 12, 13, 14, and 15 from *Science*.

than the millilambert. This is the micromicrolambert ($\mu\mu l.$) and is the equivalent of one million millionth of a lambert, or lambert x $10^{-12}$. It is very nearly equal to one thousand millionth of a foot candle. With this unit, all possible values of the threshold yield positive logarithms.

### X. PROCEDURE

The subject enters the cubicle in the dark room and sits on a chair whose height is adjusted for comfortable observation. The chin rest is arranged so that the subject's eye fits into the eye-piece and he can easily see the fixation point through the exit pupil. A few flashes of the test light are shown for illustration, and the procedure is explained to the subject. Then he is exposed to the light-adapting system. The diameter of the light-adapting field is nearly twelve times that of the test field; its area is therefore more than 100 times as great. This assures a good light adaptation for a large area surrounding the one used later for testing.

Some seconds before making the change from light to darkness, the subject is warned, and precisely after 3 minutes of light adaptation, the lever is moved and the measuring system brought into place. From previous experience we know the approximate intensity which will be visible after a few seconds of darkness, and the wedge is set at this position before the change to darkness. This intensity is flashed on every second or so, and the observer is asked whether he has seen the circle of light. He usually reports its appearance between 6 and 8 seconds after dark adaptation has begun. He then relaxes for a minute and then resumes his observing position. The wedge has been changed so as to produce a lower brightness; the light is flashed on and the subject is asked to report its presence. He usually cannot see it, whereupon the wedge is changed so as to increase the intensity. The light is again flashed and the subject says whether he saw it or not. Depending on his answer, the wedge is changed and the light flashed every 15 seconds until a point on the wedge is found above which the subject reports that he can see the flash, and below which he reports that he cannot. The subject rests for a

minute, and the procedure is repeated to determine the threshold. This is done four or five times in the next 7 or 8 minutes, thus establishing the course of the cone adaptation. The subject always reports these flashes as blue or violet. When the rod portion is reached, measurements are made once every 2 minutes on the rapid down portion, and then once every 3 or 4 minutes until the end of 30 or 35 minutes in the dark. These lights are always reported as colorless. Between test flashes, at least 10 or 15 seconds are allowed, and between the separate threshold determinations the subject always takes his eye away and relaxes. Either the subject or the recorder varies the brightness of the fixation point so that it is only just visible.

It is unnecessary to give any preliminary dark adaptation before the light adaptation. The subject always spends a few minutes in the dark room before entering the cubicle and beginning the run, and this is adequate. Moreover, the light-adapting brightness is high enough to outweigh any residual light-adapting effects that may remain from the time before the subject has entered the dark room.

At the end of the measurements, the subject has been dark adapted for at least 30 minutes and perhaps longer. It is often useful to make a rapid dark adaptation run at this point. For this purpose the light adaptation intensity is 3 millilamberts, and also lasts 3 minutes. Because the light adaptation is so low, the resulting dark adaptation is purely rod adaptation and is very rapid indeed. It is usually over in 4 or 5 minutes; it is best, however, to continue the measurements for 10 or 12 minutes. In most cases the threshold which is achieved this way corresponds almost precisely to the one achieved at the close of the previous dark adaptation.

If one should wish to determine only the final threshold, this short dark adaptation would be adequate. However, this cannot be done without first dark adapting the eye completely for at least 20 minutes. Therefore one may as well avoid this inactive period by first making the controlled dark adaptation measurements following a high light adaptation.

In making the measurements with an inexperienced subject

no great stress is to be laid on the accuracy of the first few points, because the subject is really learning how to make the measurements. The subsequent points, however, are usually very reliable. A good deal depends on the skill and experience of the experimenter. These he can acquire by practice on willing subjects. In addition it is well for the experimenter to serve as subject several times so as to realize what confronts the inexperienced person whose dark adaptation is to be measured.

## XI. SUMMARY

1. A short description is given of the structure of the retina and of the nature of dark adaptation in which it is shown that in order to measure dark adaptation adequately there must be specified the intensity and duration of the preadaptation light, and the area, the retinal location, the color, and the duration of the measuring light.

2. An apparatus is presented which incorporates these specifications, and a procedure and technic are detailed for using the apparatus under definite conditions.

3. Measurements of a normal, well-nourished population show that the limits of ordinary healthy variation in dark adaptation are not wide. The final rod threshold intensities vary within a factor of about 2.5, while the final cone thresholds cover a smaller range.

4. Healthy individuals deprived of vitamin A show an almost immediate rise in the intensity level of their dark adaptation. After a week of deprivation the intensity level is well above that found in an ordinary population, and may be diagnosed with reasonable certainty. After 4 weeks of deprivation the intensity level of dark adaptation may be 100 times as high as normal. Resumption of a normal and even of a vitamin A supplemented diet does not produce a spectacular drop in visual threshold, but rather a slow return to normal lasting several weeks.

5. People with alcoholic cirrhosis of the liver show large changes in dark adaptation comparable to those found in prolonged avitaminosis. Treatment with vitamin A in large doses brings the course of dark adaptation back to normal.

6. It is suggested that with care, dark adaptation may be used as an aid in the diagnosis not only of avitaminosis A produced by a lack in the diet, but by a failure of some organ in the path of the flow of vitamin A from the diet to the retina.

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# THE USE OF THE PHOTOMETER IN DETECTING LATENT AVITAMINOSIS A

JACOB B. FELDMAN[1]

For the past seven years I have been interested in the subject of photometry and dark adaptation. At first my work on this subject was particularly devoted to ocular diseases of various kinds, especially glaucoma, and retinal arteriosclerosis; later my interest extended to such conditions as vitamin A deficiency. My results on pathologic photometric studies may be arbitrarily divided into two main groups. In the first, or organic group, are found some cases of glaucoma, arteriosclerosis, choroiditis; included in this group are also all types of retinitis pigmentosa. In the second, or functional group, are found cases of latent avitaminosis A. The photometer, in many cases of glaucoma, does offer a means of early diagnosis; but it is by no means entirely reliable in detecting all cases, since normal photometric measurements have been obtained in several individuals showing increased ocular tension. Found much more consistently, on the other hand, are the abnormal readings associated with avitaminosis A. After trying to establish some etiologic connection between glaucoma and avitaminosis A, inasmuch as both gave pathologic photometric readings, I now feel that they have at most a remote relationship.

A number of photometers have been devised since Aubert, in 1865, constructed the first one by heating a platinum wire. In America, Derby, Waite, and the Harvard group, among others, constructed an instrument by which they developed a technique for studying dark adaptation. One or the other of the two photometers which I devised were used for all my photometric measurements in the present work. The large instrument is quantitative, giving results in millilamberts and photons. The smaller one was designed to give only qualitative results. Since most of

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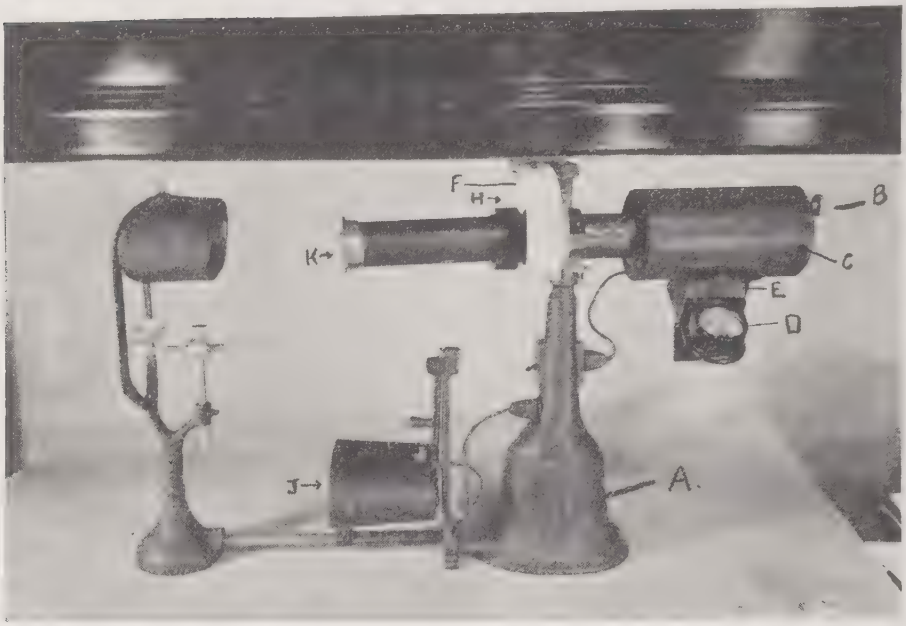


Fig. 1. Photometric apparatus for measurement of dark adaptation. Explanation of labeled parts is detailed in text.

my observations were made with the larger instrument, the more detailed description will be given of it (Fig. 1).

In the base of the larger instrument, partly to steady it because the tests are made in a dark room, there is a volt control *(A)*. Its purpose is to control the voltage of the apparatus, regardless whether an X-ray or several elevators in operation are drawing current from the main circuit at the same time. The voltage is always constant at 6 volts. Every one of the lamps used in the apparatus is seasoned and carefully selected. Before a test is made, a knob *(B)* in the lamphouse *(C)* is turned until it registers 20 on a dial. A photometer *(D)*, inserted in case *(E)* should then read 22 foot candles. This assures a constant unit of electricity in the machine, and a constant intensity and brilliance of light.

Insertion of a plain piece of paper into the holder *(F)*, which is much like the vise for holding the blade in a Gillette razor, and tracing a line across the paper produces the scratch mark from which the reading will begin. By pulling down the turntable, the paper is divided by a pencil line for the readings of the right and left eye.

During a test, the intensity and quality of the light are con-

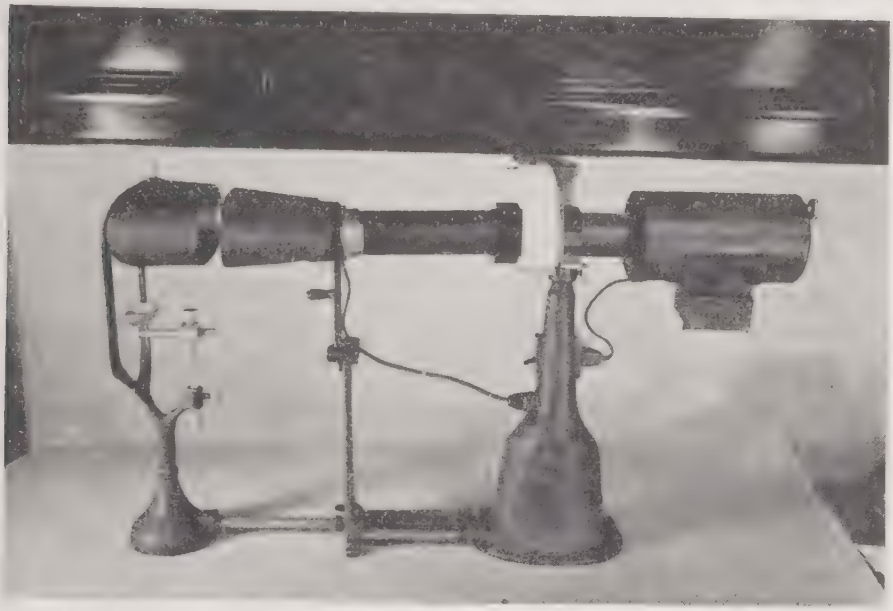

Fig. 2. Photometric apparatus as in Figure 1 with preexposing chamber in position for use.

trolled by the use of a diaphragm attached to the turntable, and when it is particularly necessary, a filter *(H)* may be used to alter the light intensity.

The "preexposure," the light adaptation prior to the dark adaptation, utilizes a light of about 6,000 photons from the lamphouse *(J)*. Figure 2 shows the apparatus with the preexposing chamber in position for use. Adaptation to the light is carried on for three minutes, after which time this section of the apparatus is pushed out of the way (Fig. 2). Although I used, at first, a small white or red light for fixation, I now make use of the kinesthetic sense in the test subject. This keeps the subject from falling asleep and also makes him share the responsibility of the test. The subject is told to look at his finger after it has been placed on a projection *(K)*. If the reading is to be with the right eye, his finger is put on the left side; if the reading is to be with the left eye, his finger is put on the right side. The observer is able to see that the subject keeps his eyes continuously opened for the specified three minutes of "preexposure." This ensures all cases obtaining the same amount of intensity of light in order to bleach the visual purple.

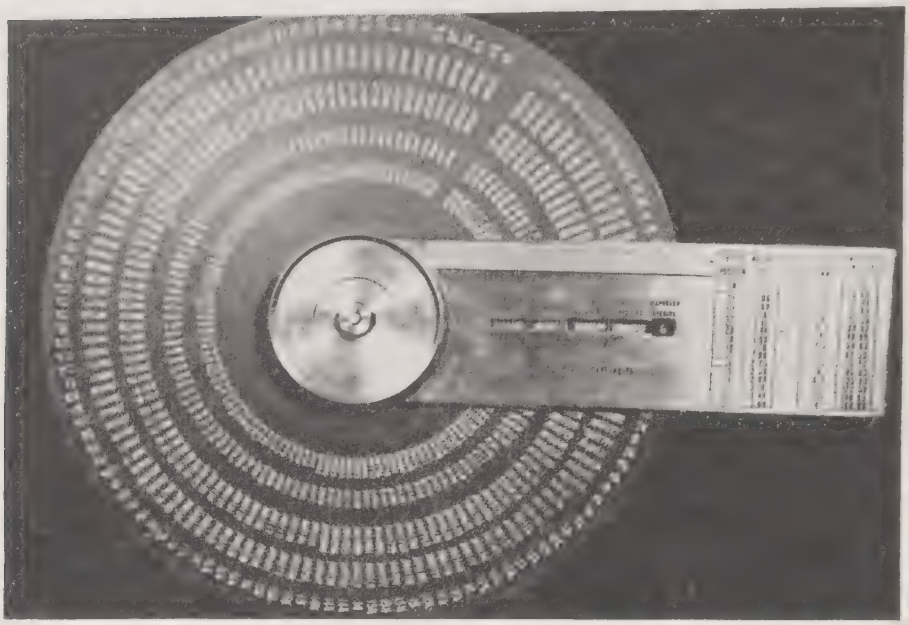

Fig. 3. The roulette wheel from which markings of the thresholds taken during dark adaptation are transposed into millilamberts and photons.

The stimulus of light is adjustable in any direction so that readings may be obtained of any particular retinal area. Usually, the area 20 degrees from the macula is studied.

The routine procedure for testing dark adaptation with this instrument is:

1. Complete ophthalmoscopic examination; field, and tension tests when necessary.
2. One drop of pilocarpine hydrochloride (1 per cent), instilled every 15 minutes for 45 minutes.
3. Measurement of pupil.
4. Patient is "preexposed" to light *(J)* for 3 minutes.
5. Light is extinguished: *subject is kept in dark for 3 minutes.*
6. Then light threshold studies are begun and taken every 3 minutes.
7. Studies are usually made for a period of 30 minutes; each eye being studied separately.
8. Markings of thresholds on the white paper on turntable *(F)* are then evaluated by the table on the "roulette wheel," illustrated by Figure 3, where results are given in both millilamberts and photons.

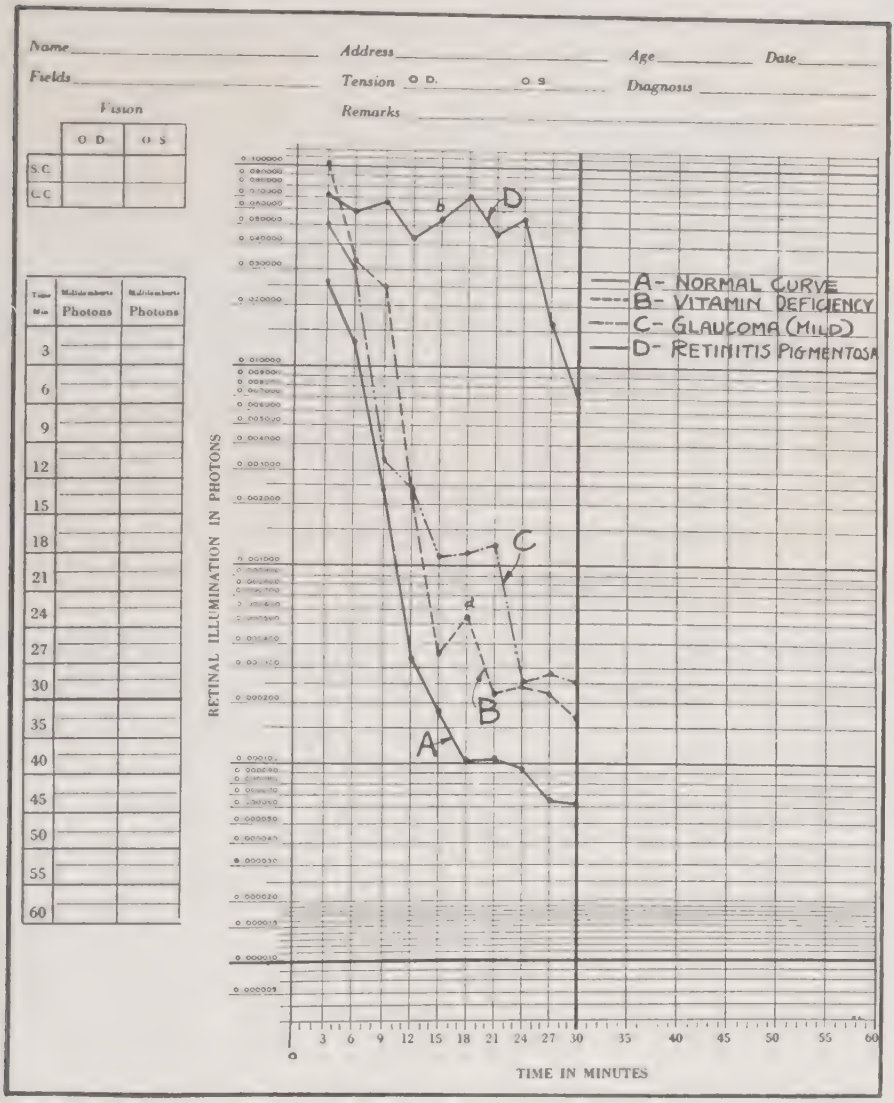


Fig. 4. Various types of actual graphs obtained during dark adaptation study. Curve *A* shows a normal response; *B* typifies the response in vitamin deficiency; *C*, in mild glaucoma; *D*, in retinitis pigmentosa.

9. Curve is plotted as on Figure 4.

With this apparatus, I have personally done 4,000 dark adaptations, the patients' ages ranging from 6 to 82 years. Examinations were made at the patients' home, several hospitals, and my office, with equally valid results. Patients examined were from all sections of the City. A large number of nationalities were

represented. Also many normal individuals were included.

In my attempt to establish a proper and simple technique, photometric measurements were taken at various minute intervals, usually for the duration of a half hour. On several occasions the study lasted a full hour. On one occasion, I found it necessary to study a case for as long as 4 hours.

There were four definite types of curves obtained during these studies (Figure 4). Curve *A* shows a normal response; *B* typifies the response in vitamin deficiency; *C,* in mild glaucoma; *D,* in retinitis pigmentosa. Final threshold readings up to .000100 photons are normal; readings up to .000150 photons are very high normals; readings above .000200 photons are slightly pathological and denote beginning vitamin A deficiency. Final threshold readings up to .000500 photons are from individuals with the extreme vitamin A deficiency and are also often seen in some cases of glaucoma, arteriosclerosis, diabetes mellitus, and hepatitis with jaundice. Final readings above .004000 photons are from the very marked cases of glaucoma and retinal diseases, such as retinitis pigmentosa. All threshold readings above .025 photons designate cone function below rod efficiency.

The lack of sensitivity at 18 minutes, as shown by the slight upturn *a* in curve *B*, with the very much increased sensitivity at 20 minutes, definitely points to inattention on the part of the patient. In contrast, the diminished sensitivity at 15 minutes, as shown by the upturn at *b* in curve *D,* with still more diminished sensitivity at the succeeding reading of 18 minutes, denotes definite pathology. I have called this "rod suppression." This is a subjective test having objective qualities.

Another characteristic points to the reliability of the photometric test. Occasionally we have found that repeating the examination, with an interval of several minutes' rest between the two, resulted in improved readings by the normal eyes in the second test. It was almost axiomatic that an eye giving satisfactory readings improved with experience, while a pathological eye became worse on the next test through fatigue. Usually in multiple tests on the same individual, the normal eye, regardless of the many tests taken, may vary somewhat; yet always remain

normal. The reverse, of course, was true in the pathologic case.

A pathologic photometric reading in the absence of ocular pathology points to a vitamin A deficiency as being the probable underlying cause. I have examined a number of ocular conditions which might be thought to affect the photometric study, such cases as patients with all varieties of squint, amblyopia, color blindness, nystagmus, conical cornea, dislocated lens, opacities on cornea, lens or vitreous, and all types of refractive errors. With the singular exception of a few very high myopias, none of these conditions materially affected the photometric reading in the individual case. Since keratinization of mucous membranes interferes with normal functions, it was felt that dysadaptation[2] might be associated with sterility. Photometric measurements of 5 sterile women, otherwise apparently normal, were absolutely normal. Several cases of exogenous obesity gave normal readings, as did 2 cases of non-toxic thyroid adenoma. Neither have I noted any photometric change as the result of increasing age without disease, or in the presence of such grave diseases as syphilis or gonorrhea.

It is interesting to note that the greatest pathology in dark adaptation was obtained in cases of myxedema. Readings on individuals with toxic goiter indicated a lesser disturbance of dark adaptation; while in several doubtful goiter cases with moderately high basal metabolism rate, the test gave normal results.

Since the liver is regarded as the storehouse of vitamin A, 4 cases of hepatitis were examined. It was expected that all would show a pathological dark adaptation; however, only 2 of the 4 cases gave a high photometric reading. Cases taken when jaundice was present gave pathological readings. A small group of 8 diabetics were studied; only 4 showed dysadaptation. It is possible that the inclusion of butter, eggs, and vegetables in the well-balanced diabetic diet might explain the normal photometric readings in the cases obtained.

That avitaminosis A causes a keratinization of the mucous membranes has been stressed by Wolbach, Howe, and others. Conceivably this cornification may form a nidus for infection and

[2] Dysadaptation means pathological dark adaptation.

thus cause renal calculi. These considerations created our interest in the photometric study of this condition. Of the 25 cases of renal calculi from the Urologic Clinic of Dr. William J. Ezickson at the Pennsylvania Hospital, 24 gave a pathological photometric reading. These patients then received various amounts of vitamin A concentrate, ranging from 13,000 to 52,000 international units by mouth, daily for 6 to 9 months. Of the 15 cases who returned for restudy, a pathological graph was still obtained in all but 1 case. If avitaminosis A is a cause of renal calculi in man, as contended by some, it would seem that either there was a failure of the liver metabolism for vitamin A, an increased rate of destruction in the body tissues and blood, or poor intestinal absorption of vitamin A in the above group of cases. Possibly some unknown toxic factor may be the underlying cause. With these possibilities in mind Dr. Ezickson and I have now completed the initial study of 35 new cases. An entirely different approach to the treatment with vitamin A concentrate has been carried out with particular reference to liver metabolism and intestinal absorption. In a few months we expect to reexamine the patients and then report the results.

Among the cases examined were 5 diagnosed as having avitaminosis A. These all show pathological graphs. To 3 of these 5, I had the opportunity of administering vitamin A. A recheck showed them to be improved in nightblindness, and at the same time the photometric reading became normal. We have noticed this curious thing, that nursing mothers, after 4 months, and pregnant women, after 6 months, often showed a vitamin A deficiency.

The instrument which was described is a quantitative device; the study takes at least one-half hour; and the plotting of the curve, an equal amount of time.

Where it is necessary to examine large groups, I use a qualitative apparatus (Fig. 5). This consists of two lamphouses *(A)*, for "preexposure," and *(B)* for the study of the light threshold. Only one reading is here depended upon as against ten threshold readings of either eye in the quantitative device. This smaller instrument is similar to the larger one in principle but dissimilar in the


Fig. 5. Instrument for rapid test of dark adaptation.

mode of measurement. While the larger apparatus measures the threshold of light intensity over a period of time, the smaller apparatus measures time for a given threshold of light, the intensity of which was selected arbitrarily on the basis of experimental data. The distribution curve of the data on 107 boys, tested by the smaller instrument, showed the greatest frequency of response between 1 and 2 minutes. Regarding a 100 per cent deviation

from this as at least a reasonable allowance, we have set the critical point of time, for test purposes, at 5 minutes. Detection of the light and its direction within 5 minutes is regarded as normal; a longer time requirement is regarded as indicative of dysadaptation.

The detailed technique for using this instrument is:

1. Complete ophthalmologic examination.
2. Three minutes "preexposure" of the patient's eyes to light from *(A)*, shown in Figure 5.
3. Extinguishing the light in *(A)*, automatically illuminating the light threshold chamber *(B)*.
4. Place patient's finger at *(C)* where it is desired he should look.
5. The patient is asked to say "stop" as soon as the light at *(B)* is seen.

The light (horizontal beam) in Figure 5 may be turned in a direction unknown to the patient. The patient must not only tell when he sees the light, but also the direction it takes. This acts as a check upon the veracity of the patient. Up to 5 minutes to recognize the light is considered normal. Above 5 minutes is pathological, and in the absence of ocular pathology is considered as evidence of avitaminosis A.

Over one thousand dark adaptations have been done with this apparatus. Comparison of results from the quantitative apparatus and those from the qualitative apparatus showed a consistency in 95 per cent of the cases.

At Wills Eye Hospital, 116 cases were examined with a pathological dark adaptation detected in 41 per cent. This high figure is probably due to the ocular pathology predominating in the sample. At the St. Christopher's Hospital for Children, located in the poorer section of the City with many children on relief, 313 were examined with pathological dark adaptation registered by 19 per cent.

At a correctional institution for girls and women, two separate tests were made: of 186 subjects examined, 11 per cent showed pathological dark adaptation; of 87 others, 16 per cent showed pathological dark adaptation. This low prevalence of pathologi-

cal dark adaptation may no doubt be attributed to the good food and excellent care these inmates were receiving. After the 20 girls with pathological dark adaptation, in the first group, were given a high vitamin diet plus 20,000 units of vitamin A daily for three weeks, 2 still remained pathological.

Of 110 boys in an orphanage, only 2.7 per cent showed pathological dark adaptation. Of the normals it was found that the greatest majority, about 85 per cent, saw the beam of light and its direction within 3 minutes. These results formed the basis of setting the critical time for the qualitative apparatus.

Of the entire 1,000 dark adaptation tests in all series, pathology in the light threshold was noted in 18 per cent (Table 1).

As has been noted before, the results from the qualitative apparatus compared favorably with those from the quantitative apparatus.

Much has been said about nightblindness as a cause of accidents. Practically everyone agrees that latent nightblindness or nyctalopia is a manifestation of vitamin A deficiency. Among our cases were 2 men who gave a high photometric reading on the smaller apparatus, indicating pathology. Both of these had been in accidents at night. Questioning elicited the fact that one could not even see the white horse with which he collided. The other knew of his difficulty, but would not tell his employer for fear of

Table 1. Results of dark adaptation tests, by either quantitative or qualitative apparatus, on individuals with various pathological conditions.

| *Normal threshold* was obtained in: | *Pathological adaptation (dysadaptation; high threshold)* was obtained in: |
|---|---|
| Alcoholism (11 cases) | Acute Severe Infection |
| Arthritis (knee) | Arteriosclerosis |
| Asthma | Cervical Adenitis |
| Choked Disc (1 case) | Choroiditis |
| Slight Cold and Hay Fever | High Myopia (3 cases) |
| Tonsillitis | Hypertension and Diabetes |
| Drug Addict | Hypertension and Kidney Disease |
| Gonorrhea (7 cases) | Microscopists |
| Infected Finger | Nursing Mothers Past 4 Months |
| Interstitial Keratitis | Pregnancy Past 8 Months |
| Low I.Q. (90 cases) | Renal Calculi |
| Sclerosing Keratitis | Sinus Disease |
| Squint (All Types) | Toxic Goiter |

being discharged. All of the patients having night blindness complained of photophobia. The connection of vitamin A deficiency and nightblindness has been stressed of late for their possible role in the number of accidents on the roads after dark. By the routine use of the photometer as part of the test for automobile drivers, airplane pilots, and railroad engineers, one might be able to reduce the ever-mounting toll from accidents.

The photometric test is subjective in nature though it has objective qualities. Shortcomings, however, may be expected at times. Much depends upon the technician. Many oculists state that they cannot obtain the normal blind spot in certain individuals, yet they do not discard the perimeter on that account. Anyone using the photometer should strive to establish skill in technique, not only in the matter of manipulating the apparatus, but also in the manner of approaching the individual under test. When this is accomplished, greater accuracy is obtained in results. Results then become reproducible. Those patients whom I have examined more than once have shown throughout, with very few exceptions, almost exactly the same values. Where the initial readings were slightly higher on one eye, the succeeding readings were, in fact, identical.

Some of the seemingly inconsistent results obtained in the past on photometric tests may not be entirely explainable on a technical basis. It is quite possible that during the several days which may have elapsed between dark adaptation studies on the same individual, his vitamin A level may have altered. This may account for a different subsequent photometric reading. A chemical test for the concentration of vitamin A in the blood would be invaluable if it were available, for it could be used conjointly with, and as a check on, the photometric test in specific cases.

ACKNOWLEDGEMENT

I wish to express my appreciation of Dr. Selig Hecht's advice on several technical points.

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## DISCUSSION

Dr. Carroll E. Palmer: In general, most of our attempts to measure the prevalence of nutritional deficiency have been by means of techniques which do not permit one to say specifically what is the matter, nutritionally, with an individual. The dark adaptation test for vitamin A deficiency has great importance from a practical point of view, therefore, because it represents, I believe, the first time that we have attempted to develop a technique that could be used for studying, on a large scale, the prevalence of a specific nutritional deficiency. In addition to the hope that we might determine vitamin A deficiency by it, the dark adaptation test may be of service in another practical way. I should think that we could speculate that a vitamin A deficiency might intimate a level of deficiency in other things besides vitamin A. Certainly, if an individual has a vitamin A deficiency, it is not unreasonable to suppose that he might have also other vitamin deficiencies. Furthermore, associated with a vitamin A deficiency there may conceivably be a deficiency in quantity of food, so that if the dark adaptation test could be entirely satisfactory as a test for vitamin A deficiency, it might serve also as a screening technique that might be a great deal better than any we have developed so far for picking up general nutritional deficiencies.

Our own work with the biophotometer was directed primarily toward a repetition of Dr. Jeans' work. As you know, by use of a biophotometer he reported a prevalence of vitamin A deficiency which ranged in children from 20 to 70 per cent. Our study consisted first of a survey of a school population. We tested about 500 children, and then picked out the 100 children who gave the least satisfactory readings on the biophotometer. Then, in order to get some kind of control of the effect of the administration of vitamin A, we subdivided the 100 children into two groups, 50 in each. The children in one group received a vitamin A supplement for a period of 8 weeks. Those in the other group, so far as they knew, also received vitamin A; actually, however, they were given capsules containing only mineral oil. At the end of 8 weeks we were unable to distinguish between the two groups of children with respect to their adaptation reactions; the children in both groups showed a very marked improvement in their biophotometer readings. We have attributed this improvement to a learning of the test by the children. It has been reported that the learning factor is not very important. I think if

one looks at reports on that subject it will be found that most of them are on adults. Our experience with children, at least, indicates that there is a very marked learning of this subjective test, and that one must be very careful in distinguishing between the effect of vitamin A administration on dark adaptation, and a simple learning of the test.

It seems unnecessary to say that we failed entirely, or almost entirely to confirm Dr. Jeans' work. Various reasons might be suggested as to why we failed. In the first place, standards for diagnosing vitamin A nutrition are very incompletely defined—so incompletely defined, in fact, that we question whether or not any of our 100 children were actually deficient in vitamin A.

At the present time, it is obvious that no general agreement exists among the representatives of various disciplines of science regarding the relationship between the dark adaptation reaction and the status of vitamin A nutrition. From a rational standpoint it seems that there ought to be some sort of relation. The whole matter requires careful and critical study.

Dr. Frederick F. Tisdall: Some 4 years ago when Jeans reported that by means of the biophotometer he found from 50 to 75 per cent of the Iowa children giving evidence of a lack of vitamin A, we felt that those findings of his should certainly be investigated. Dr. Charles E. Snelling of the Department of Pediatrics, University of Toronto, investigated the matter for a year or longer, taking a group of school children in a boarding school. After he had done his initial examination, he gave one-half of them vitamin A, and repeated the test. The conclusions, worded carefully, were these: In our hands, this machine has been unsatisfactory for the estimation of small variations in dark adaptation such as might be produced by vitamin A deficiency.

Shortly after that Jeans came out with another paper stating that in his hands the machine had not proved entirely satisfactory, and recommended another machine.

I will give you briefly some of the recent results with this new machine which Dr. Snelling has obtained in Toronto. He took approximately 30 children from the Out-Patient Department, such as we would obtain if we were going to make a dietary survey of the community. They were just the ordinary run of children, coming into the Out-Patient Department, not seriously ill.

He ran through the routine test as outlined by Jeans. Then the

test was repeated from 1 to 3 weeks later, when it was found that almost 50 per cent of those children had a different reading on the second test than on the first, although there had been no change in the routine at all. To take the exact figures, out of the 30 children, 17 remained the same, 5 improved, and 8 became worse. That means that 13 out of the 30 had changed, although there had been no change in the treatment of the children at all. Then, for the next 3 weeks he gave all these 30 children approximately 14,000 units of vitamin A daily, and then tested them again. Eighteen of them remained the same, 7 improved, and 4 became worse.

Now of these 18 that remained the same, if they were not suffering from any vitamin A deficiency we would expect them to remain the same, because an additional amount of vitamin A over what is needed would not produce any change. But when he went back and examined his initial findings of these 18, he found that 10 were definitely abnormal, and 3 were borderline cases according to Jeans' technique. Theoretically, if those 10 abnormally low cases and the 3 borderline cases were due to a lack of vitamin A, we would have expected some improvement of those 13 cases.

So in summarizing we can say that, first, with no change in their treatment, almost 50 per cent of the children gave different results on the second test from the first test, and secondly, after giving vitamin A, about 14,000 units daily, 4 of the cases became worse, 7 improved, and 18 remained unchanged, 10 of the 18 being definitely low and 3 of the 18 being borderline cases on the first test.

Dr. Arthur F. Abt: I should like to ask if anyone has had any experience with clinical cases where fat absorption was definitely poor, such as in choluric jaundice or in celiac disease. Have such cases been tested by this method?

Dr. Elaine P. Ralli: So far as the experimental animal is concerned, there is a fatty infiltration of the liver. We made a test on animals and had a piece of liver removed at one time, and another piece removed at intervals of 90 to 150 days. There was a definite loss of both vitamin A and carotene in the liver, and at the same time an increase in excretion of the fat.

This, of course, is not the same as in children; but that particular test we have done, and found that with the real interference with fat utilization—perhaps it would be wiser to say that although abnor-

malities must enter into it, there is some change in the metabolism through vitamin A, as far as the liver is concerned.

Dr. Selig Hecht: Someone has asked whether pregnancy has any effects on the vitamin A content of the body. We have made no measurements ourselves, but Edmonds in Copenhagen reports that pregnant women who have had difficulty with their eating, who have had frequent vomiting attacks, showed disturbances in vision which were corrected by the administration of large amounts of vitamin A. However, it is fairly likely that this vitamin A lack was not due primarily to the pregnancy, but to the failure to take enough food.

In commenting on the variously reported inconsistent results with the "biophotometer," I can hazard the opinion that the failure lies in the method of use of that instrument. After light adaptation to the low brightness of about 75 foot candles, the subject sees 5 dots, each occupying about 1 degree visual angle. What one is expected to observe is the central dot, and one must change the illumination until this central dot disappears.

This results in a paradoxical situation. If one really looks directly at this central dot, one is measuring only cone dark adaptation, because the dot is only a 1 degree field. Thus, if one looks carefully at this central dot, one stops dark adapting very rapidly because one is measuring with the cones only, and as you already know, cone adaptation is very rapid and is over very soon. But if one does not look at the central dot directly, but naturally tries to pick it up with the corner of the eye, one adapts for a longer time because one is using the rods of the retina. The poor patient makes the best of the situation, and uses now the center of the eye, and now the corner of the eye. It is this which probably causes a large degree of uncertainty. The instrument thus makes one strike a compromise position, which one learns to duplicate to a certain extent.

There are many other structural errors in the biophotometer, but it would take us far afield to enumerate them. One of the interesting ones is that it uses a rheostat to change the intensity of the measuring light by varying the amount of current going through it. As a result the color of the measuring light is changed, and one records what happens to the instrument as well as what happens to the eye.

In all these studies the need for concurrent chemical researches is all too clear. We should have not only the statement that this person improved in visual behavior after vitamin A administration,

but also the results of a concomitant chemical test which shows that the vitamin A content of the body has been changed.

The point raised about the possibility of detecting latent, subclinical avitaminosis A by means of a visual test, can be answered with a reasonable optimism. The chances are high that with a good instrument and a good technician, one can say that a given individual has, for example, a dark adaptation curve or a final threshold which is 5 times higher than what one finds normally in the population. Therefore, such a person had better be examined carefully. He may have a stone in the kidney, he may be suffering from cirrhosis of the liver, or from just plain malnutrition, but at least the dark adaptation has given us a diagnostic sign to go by.

Age is not an important factor in dark adaptation. In our survey of 110 individuals between 14 and 65 no significant differences in adaptation were apparent with age, except that older people tend to show a higher cone threshold.

The general impression seems to be that the threshold of dark adaptation returns to normal rapidly with vitamin A administration. Our 4 cases do not show this, and we are doubtful of the validity of this general impression.

The final point raised was with regard to diseases like jaundice in which biliary materials come into the blood. Visual purple and visual violet (the rod and cone pigments) happen to be peculiar in that they are soluble in bile salts. Therefore, one of the effects of jaundice and similar diseases is probably a direct action of the circulating bile salts on the retina; the visual pigments may actually be bleached out of the rods and cones.

Dr. Jacob B. Feldman: In my qualitative instrument a single reading is obtained of the threshold as against 20 threshold readings on the quantitative device. I do not use the qualitative device to the exclusion of the quantitative apparatus. Where large numbers of subjects are to be examined, however, it was deemed preferable to use the qualitative device. It is a time saver and was found quite reliable when results were compared with those of the quantitative machine.

In the average case, a normal threshold is obtained by the qualitative device in 2½ minutes; permitting up to 5 minutes gives an allowance of 100 per cent to still be classed within the normal range.

From the literature on vitamin A, I understand that in some

instances the vitamin content in the blood has nothing to do with the vitamin content in the body or in the liver. I think, therefore, that even a blood test would only be an aid, but not a positive criterion as to the vitamin A content of the individual examined.

# THE APPLICATION OF ELECTROCARDIOGRAPHY IN THE DETECTION OF AVITAMINOSIS $B_1$

SOMA WEISS[1]

THE important role of deficiency of vitamin $B_1$ in the causation of certain clinical syndromes is well known. Further advance in this field is hampered at present mainly by the lack of specific and reliable tests for the qualitative and quantitative measurement of vitamin $B_1$ in body fluids. The chemical or biological tests applied are cumbersome and often not adequately reliable. Among the indirect methods in use is the registration of electrocardiographic changes.

## CARDIAC CHANGES IN ANIMALS

In 1930, Drury, Harris, and Maudsley (1) observed a slowing of the heart rate in rats maintained on diets deficient in vitamin $B_1$. This bradycardia was specific for vitamin $B_1$ and has since been used as a test for the estimation of the vitamin $B_1$ content of various substances, tissues, and body fluids (2). The cardiac slowing observed in rats is not a regular manifestation of vitamin $B_1$ deficiency in all species of animals since some of the higher mammals, including man, do not exhibit it. Man develops tachycardia rather than bradycardia in conditions associated with vitamin $B_1$ deficiency. Furthermore, the intrinsic cardiac mechanism of the bradycardia is apparently not always the same. Thus, the bradycardia observed in the rat depends on changes in the myocardium and is not abolished by section of the vagi, while that occurring in the pigeon is abolished by vagotomy.

The majority of investigators who have applied the electrocardiographic method for the detection of vitamin $B_1$ deficiency have utilized this technique primarily for the measurement of the cardiac rate and have failed to use standardized leads. Carter and Drury (3) and Méhes and Péter (4) have observed heart block

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in addition to bradycardia in pigeons fed on polished rice. Méhes and Péter (4) also observed slight electrocardiographic changes in some of the rice-fed pigeons, consisting in occasional prolongation of the P-R interval and lowering of the S and T-waves.

Recently we have succeeded in obtaining standardized electrocardiographic leads on the rat. We have made a study of the cardiac rate, electrocardiographic complexes, and the response to drugs in rats in the nondeficient state and in rats with repeatedly induced vitamin $B_1$ deficiency (5). The results of this study indicate that the heart rate of rats on a diet deficient in vitamin $B_1$ fell gradually to a level of from 300 to 350 beats per minute, from which it usually returned to approximately normal (450 to 500) with adequate subcutaneous doses (5 to 25γ) of crystalline vitamin $B_1$, even if food was withheld (Fig. 1). There was some relationship between the onset of critical cardiac slowing and neurological manifestations of vitamin $B_1$ deficiency. In all but 4 or 5 of the 22 vitamin $B_1$-deficient rats studied the decrease in heart rate was accompanied by changes in the electrocardiographic complexes, consisting most frequently in an increase in height, flattening, inversion or high or low take-off of the T-waves and depression of the S-T segments (Fig. 2). With adequate doses of vitamin $B_1$ the T-waves usually returned to normal within several hours or a day, although occasionally several days were required. The changes in the electrocardiographic complexes had no close relation to the level of the heart rate and were not identical in the same animals on successively induced deficiencies. With the exception of very low cardiac rates, the P-R interval remained essentially unchanged. The ratio of the Q-T interval to the square root of the R-R interval (K) usually did not increase as the cardiac rate decreased.

It is well known that the cardiac manifestations of beriberi in man are apt to be precipitated by physical exertion. An attempt was made, therefore, to ascertain whether the cardiac changes described above occur sooner or are more marked in animals trained to exercise on running wheels. Only one of four rats allowed to exercise on running wheels became deficient sooner than other vitamin-deficient animals. In none of the four exer-

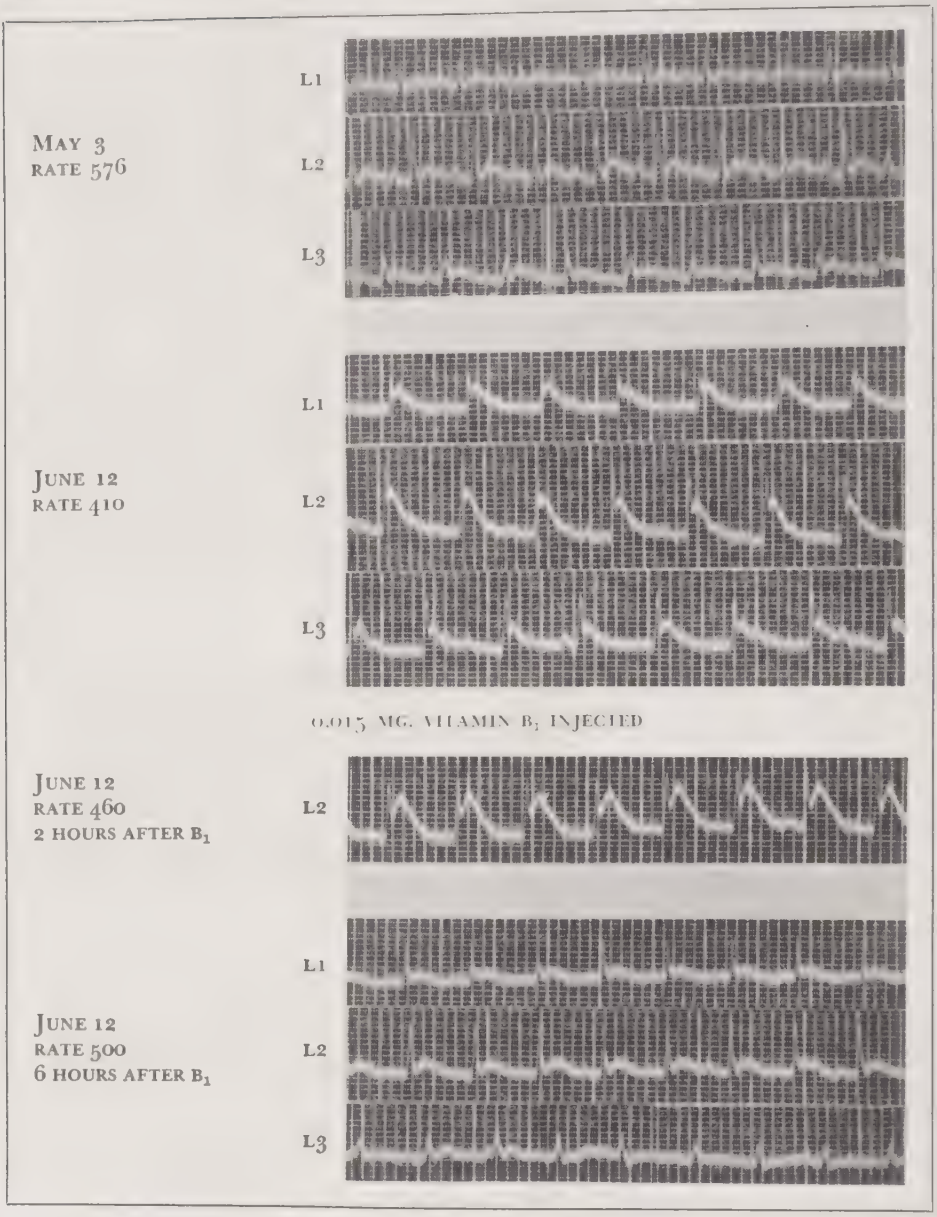


Fig. 1. Electrocardiograms on a rat fed a diet deficient in vitamin $B_1$, before and after the injection of crystalline vitamin $B_1$. The time lines are 1/50 second apart. Note the increased height and the high origin of the T-waves on June 12. Six hours after the administration of vitamin $B_1$ the T-waves were again practically normal.

cising rats were the cardiac changes more marked or of different character than those found in control vitamin-deficient rats.

The comparative effects of certain drugs on the cardiac rate

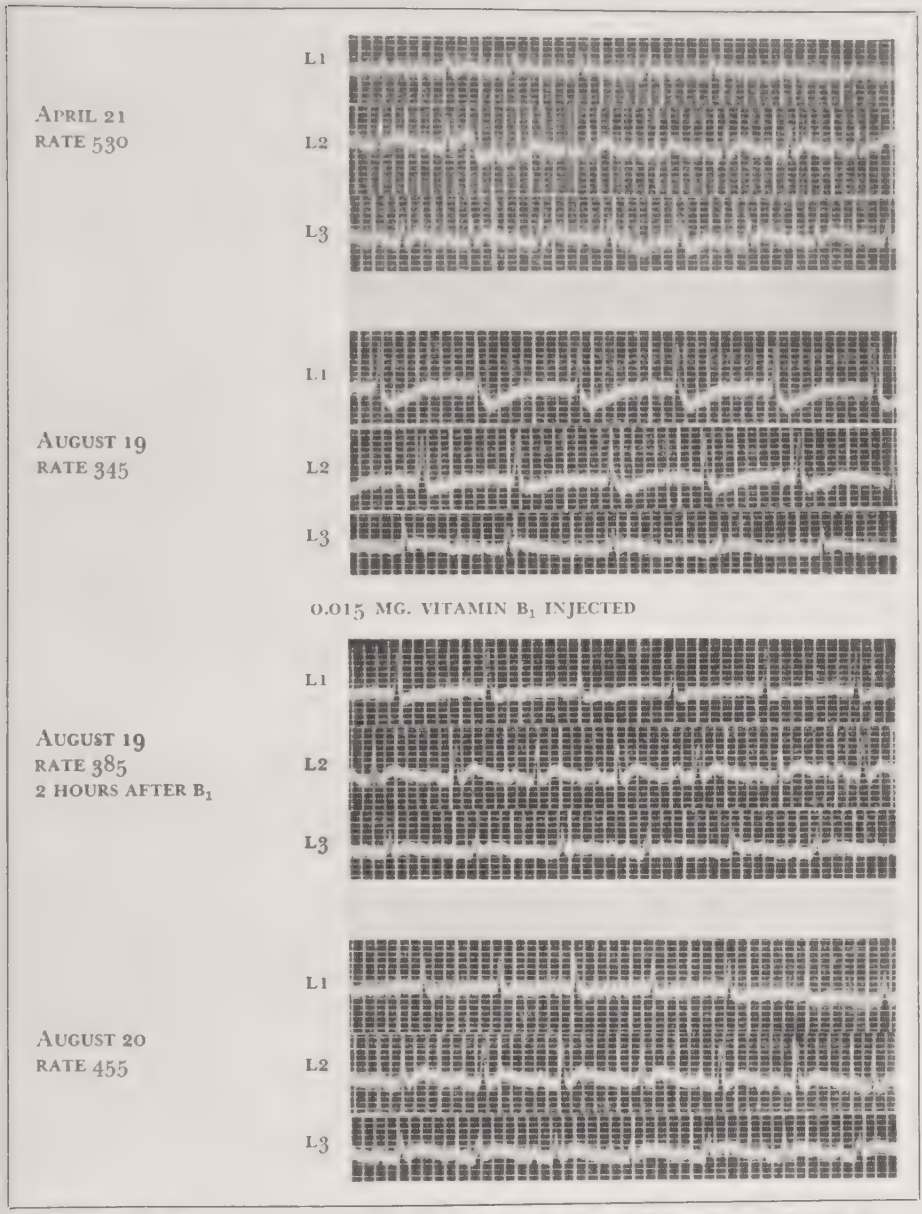


Fig. 2. Electrocardiograms on a rat fed a diet deficient in vitamin $B_1$, before and after the injection of crystalline vitamin $B_1$. The time lines are 1/50 second apart. Note the changes in the T-wave and the depression of the S-T segment on August 19 and the normal complexes after vitamin $B_1$ was given.

and on the electrocardiograms of normal and deficient animals, respectively, have also been studied. The cardiac responses of normal and of vitamin-deficient rats to epinephrin were essen-

tially the same. Occasional irregularities were observed in the vitamin-deficient animals. Atropin and section of the vagus nerves did not abolish the cardiac slowing or the electrocardiographic changes produced by vitamin $B_1$ deficiency. The vitamin-deficient rats were more sensitive to the toxic effects of subcutaneous doses of strophanthin, and depression of the S-T or inversion of the T-wave occurred with doses which caused no change in normal rats.

More recently, Haynes and Weiss (6) have extended this study. It has been shown by several investigators that in experimental vitamin $B_1$ deficiency certain substances, such as pyruvic acid, lactic acid, glyceraldehyde, methyl glyoxal, and other intermediary metabolic products, accumulate in the body. An investigation has been made to ascertain whether the administration of some of these substances could induce in normal or in partially deficient rats the cardiac changes and electrocardiographic abnormalities described above. An answer to this question is essential in order to ascertain whether the cardiac changes depend directly on the "toxic" effect of certain substances and hence only indirectly on the lack of vitamin $B_1$. We were unable to reproduce the electrocardiographic changes characteristic of vitamin $B_1$ deficiency in normal animals even with reduced food intake. Deficient rats have received large doses of these substances without marked effect, with the possible exception of sodium pyruvate. Even with this substance the electrocardiographic changes were not pronounced. When these metabolites were given subcutaneously they failed to prevent recovery in animals to which vitamin $B_1$ was also given. These results tend to confirm Péter's contention that the "biochemical lesions" in vitamin $B_1$ deficiency are the result of "absence of an important factor in the development of energy from carbohydrate" and do not occur "through any toxic effect of accumulated lactate or other metabolite."

### CARDIAC CHANGES IN MAN

The evidence available indicates that beriberi is caused *primarily* by deficiency of vitamin $B_1$. Like all nutritional deficiency diseases in man, beriberi seldom results from lack of a single

substance. Man rarely chooses or utilizes his food so that it is deficient in only one factor. The possible modifying influence of simultaneous deficiencies on the human body is not understood at present, but this may be one of the factors responsible for certain differences between deficiency diseases in man and experimentally induced deficiencies in animals.

Beriberi has been known for centuries as a devastating disease of the rice-eating people of the Orient. Subsequently it has been observed in other parts of the world. The "dry" form of the disease manifests itself mainly in muscle wasting and in neuritis; the "wet" form is associated with cardiovascular disturbances and edema. It has been pointed out that the term "beriberi heart" is not appropriate for the designation of the circulatory disturbances of the disease because the circulatory dysfunctions depend on disturbance both of the peripheral vessels and of the heart. The clinical characteristics, physiology, morbid anatomy, differential diagnosis and treatment of the disease, particularly as observed in the United States, have been described elsewhere (7) (8) (9) (10). Enlargement of the heart with predominant dilatation of the right ventricle associated with overactivity and tachycardia are the usual findings described in beriberi (11). As indicated, such changes occur in the more advanced cases. Tachycardia without cardiac enlargement was present frequently in milder cases.

There are mainly two explanations of the pathogenesis of beriberi heart. The older theory, advocated by early Japanese workers, claims that cardiac disease is caused by disturbance of the function and by "degeneration" of the vagus nerves (12). The histological evidence for this theory, however, is inadequate. Aalsmeer and Wenckebach (13) proposed a myogenic origin of the cardiac disturbances. They have observed a shortened P-Q conduction time in some of the patients with severe circulatory disturbances. Keefer (14) has described low voltage and negative $T_3$ waves in some of the cases observed in China. Feil (15) has observed electrocardiographic changes in a group of patients with pellagra.

In 67 cases with various cardiovascular manifestations of beri-

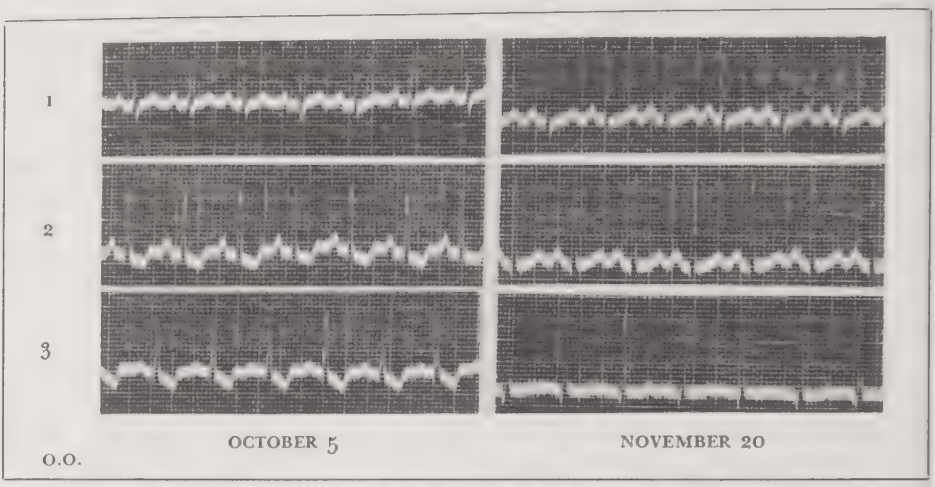


Fig. 3. Change in S-T complexes of electrocardiogram in a case of alcoholic polyneuritis before and after treatment with oral vitamin B concentrate.§

beri in which electrocardiograms were taken, abnormalities were disclosed in all but five (8) (Fig. 3). None of these patients had organic heart disease and their arterial pressure was normal. The most common changes consisted in change in direction of T-wave (93 per cent). Sinus tachycardia, with a rate of 100 or over, was present in 68 per cent of the cases. A prolongation of the electrical systole (Q-T interval) occurred in 79 per cent (Cheer Li standards) and 45 per cent (Shipley-Halloran standards) of the cases, respectively. Other changes in the electrocardiograms, such as auricular and ventricular premature beats, low voltage of the Q-R-S complexes, auricular fibrillation and intraventricular block, have also been noted. There were patients with histories of vitamin $B_1$ deficiency in whom the only detectable objective change was in the electrocardiogram. The electrocardiographic changes disappeared with improvement after a diet rich in vitamin $B_1$ or crystalline vitamin $B_1$ was given (Fig. 4). The time element of disappearance varied considerably. The factors which determine the rate of disappearance of the electrocardiographic changes are not known. Patients were also observed in whom abnormality of the electrocardiogram appeared or became accentuated soon after the administration of vitamin $B_1$ or of food rich

§ Figures 1 and 2 are reproduced through the courtesy of the *American Heart Journal;* figures 3 and 4, the *Annals of Internal Medicine.*

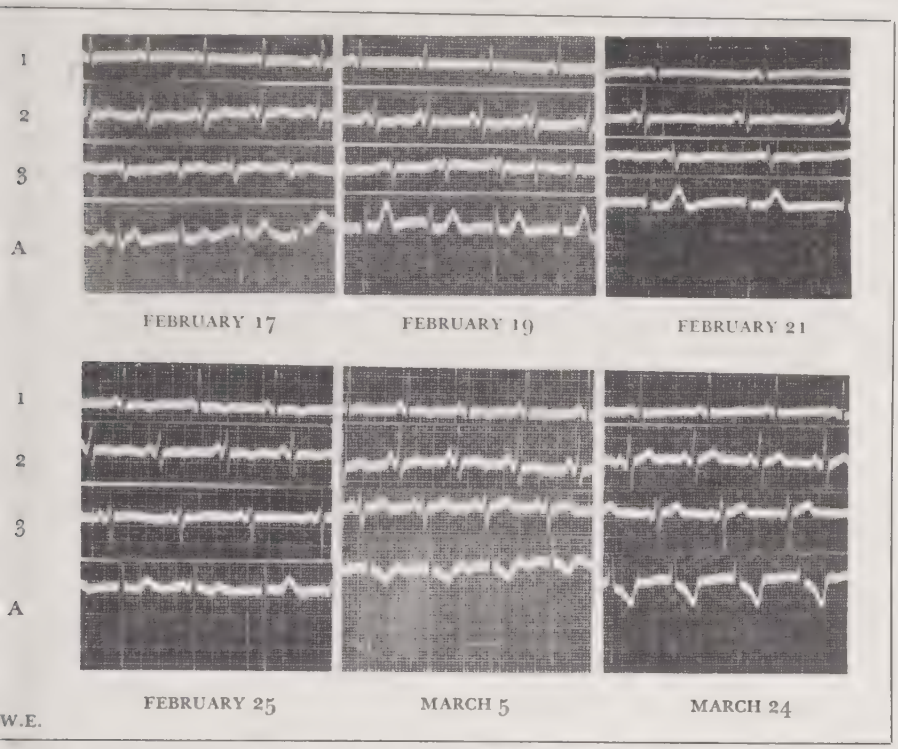


Fig. 4. Electrocardiograms in a case of beriberi with cardiac complication. Tracings obtained February 17 and February 19 before treatment; February 21 two days after administration of crystalline vitamin $B_1$. Note slowing of rate. Tracings obtained February 25, March 5 and March 24 show progression of T-wave changes toward normal (Lead A=apical lead.)

n vitamin $B_1$, only to disappear eventually, an observation also oted in deficient animals following the injection of vitamin $B_1$.

It is obvious that the electrocardiographic changes observed in nimals or men deficient in vitamin $B_1$ are not specific. Indeed, it as been stated (9) that the entire clinical characteristics of the rdiovascular changes in beriberi, as in other cardiovascular disases, are not specific. The manifestations of the disease, together ith the lack of history of other types of heart disease, nevertheess permits diagnosis with a fair degree of probability. The elecocardiographic changes which disappear after the administraon of vitamin $B_1$ represent an additional valuable and objective onfirmatory sign of vitamin $B_1$ deficiency.

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# THE STATUS OF THE COLOR TESTS FOR VITAMIN B-1

SAM Z. LEVINE AND E. MARPLES[1]

THE vitamin B complex was discovered by Eijkman in 1897, and 29 years later it was separated into at least two components by Smith and Hendrick. In 1926, the antineuritic component $B_1$ was isolated in crystalline form from rice polishings by Jansen and Donath and given the name aneurin. Williams and his associates were able in 1936 to identify the chemical structure and artificially synthesize crystalline $B_1$. By physiological assays, chemical analysis, and physical methods, the latter authors demonstrated that the synthetic and natural crystalline vitamins were identical. To their synthetic product they gave the name thiamin chloride. Peters prefers the name crystalline torulin.

The empirical formula of thiamin chloride is $C_{12}H_{18}N_4SOCl_2$ and structurally it consists of a 2-methyl, 4-amino-pyrimidine group joined to a 4-methyl, 5-beta-hydroxyethyl thiazole group by a methylene group through the 5-carbon atom of the pyrimidine ring and the nitrogen atom of the thiazole ring (Fig. 1). It occurs as colorless, odorless crystals or powder, soluble in water, acetic acid and 70 to 80 per cent alcohol, but insoluble in organic solvents such as carbon tetrachloride, chloroform, benzene, and acetone. The crystalline vitamin is not destroyed by air or light and destruction by moist heat takes place only at high temperatures (100° C.) for prolonged periods. It is stable in acid

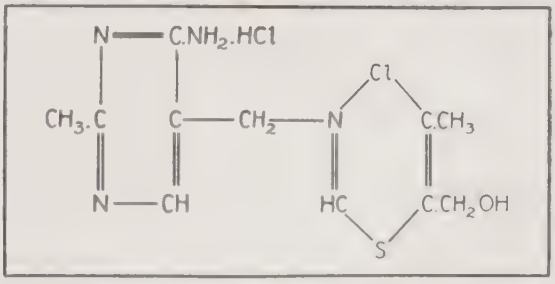


**Fig. 1. Structural formula of thiamin chloride.**

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but not in alkaline media and behaves as a base, forming insoluble compounds with picric, phosphotungstic, and tannic acid. It is quantitatively split at room temperature by neutral sulfite, nitrite, and acetate solutions. It is absorbed from neutral or acid solutions of Fuller's earth or silica gel and can be released by treatment with alkali or quinine.

For $B_1$ the International Standard of the League of Nations is an acid-clay adsorbate from rice polishings; 10 mg. of the adsorbate equalling 1 I.U. which in turn is equivalent to approximately 0.003 mg. (3 micrograms) of crystalline thiamin chloride (2-γ thiamin = 1 Chase Sherman unit = ½ Schlutz Knott unit).

The available methods of assay for vitamin $B_1$ may be conveniently divided into three groups: biological, chemical, and miscellaneous (Table 1). The biologic assays on animals have until recently proved most reliable but the newer chemical procedures offer much promise for the future.

The biological method which has been most widely used for assaying the vitamin $B_1$ content of foods is the rat-feeding or growth procedure. Briefly, this method consists of comparing the rate of growth of healthy young rats previously depleted of their body stores of $B_1$ and subsequently given weighed amounts of the dietary substance to be tested, with the growth rate of negative and positive control animals. It has given fairly consistent results (± 12.5 per cent in 2-week tests) but its specificity is open to question since there may be thermolabile vitamins other than $B_1$ in yeast and foods generally.

The rat (Dann) and pigeon (Kinnersley and Peters) curative

Table 1. Methods of assay for vitamin $B_1$.

| Biologic | Chemical | Miscellaneous |
|---|---|---|
| 1. Feeding or growth (rat) | 1. Thiochrome | 1. Catatorulin |
| 2. Curative (rat or pigeon) | 2. Diazo Reaction | 2. Mold Growth |
| 3. Bradycardia (rat) | a. Kinnersley and Peters | 3. Yeast fermentation |
| | b. Prebluda and McCollum | |
| | 3. Thiazole Method | |
| | 4. Pyruvic Acid Methods | |
| | a. Bisulfite Binding Power | |
| | b. Phenylhydrazone | |

methods are more specific but they have not yet been tried extensively for food-testing purposes. Furthermore, these methods require critical judgment for deciding the exact stage in the deficiency cycle (spastic polyneuritis) at which to administer the substance to be tested. The bradycardia method of Harris and Leong, although a delicate one, is also not generally accepted as a highly specific test for vitamin $B_1$ (its error being about $\pm$ 10 per cent).

It is recognized that all biological methods of assay are open to a number of shortcomings. They are laborious, expensive, and relatively inexact. The effect of known reference standards must be studied simultaneously on litter mates. Finally, as they pertain especially to $B_1$, there are a number of physiologically similar substances and the International Standard or acid-clay adsorbate may, because of its relatively firm combination, not yield its full quota of $B_1$ in animal assays.

Because of the recognized defects of biological assays for vitamin $B_1$ in animals, the introduction of a simple, exact, and reliable chemical method of assay, applicable to foods, tissue fluids (blood), and excreta (urine and feces) would be of inestimable value. Unfortunately, however, vitamin $B_1$ does not lend itself readily to chemical assays for the following reasons: (1) it occurs in the proportion of only 0.1 to 4 parts per million in most natural sources of food or roughly one thousand times less than vitamin C; (2) unlike vitamin C, only a small percentage of ingested or injected $B_1$ is excreted (5 to 8 per cent); (3) no quantitatively efficient method for extracting and concentrating the vitamin from its natural sources has as yet been found; (4) vitamin $B_1$ possesses no known physical property which is adapted to delicate testing; and (5) none of the methods yet devised is absolutely specific.

Despite these theoretical difficulties, a number of chemical methods have recently been proposed for assaying $B_1$ (Table 1). Briefly, they include: (1) The thiochrome method of Jansen. (2) The adaptations of the diazo reaction by Kinnersley and Peters and by Prebluda and McCollum. (3) The thiazole method of Naiman which is based on the formation of an insoluble orange-

red compound of thiazoles with bismuth or antimony in the presence of potassium iodide. This precipitate is filtered, dried, and weighed. Since this is a gravimetric method, it probably will not be applicable to the minute amounts of $B_1$ excreted in the urine or to the usual small amounts found in natural food. (4) Methods designed to estimate the pyruvic acid content of the body fluids as an indirect means of determining clinical vitamin $B_1$ deficiency. It has been shown that in the absence of $B_1$, which presumably acts as a co-enzyme, the oxidation of carbohydrate within the body stops at the pyruvic acid stage with consequent accumulation of this substance, its aldehyde (methyl glyoxal) and, secondarily, lactic acid. Two methods based on this accumulation of the intermediary products of carbohydrate metabolism (pyruvic acid and methyl glyoxal) have been proposed for testing $B_1$ deficiency: The first, dealing with the bisulfite combining power of the blood, depends on the reaction of aldehydes and ketones with this substance at room temperature to form bisulfite compounds in the presence of excess sodium bisulfite, the excess being removed from solution by adding iodine. The bisulfite compounds thus formed are hydrolyzed by saturation of the solution with sodium bicarbonate and the liberated bisulfite is titrated with dilute standard iodine solution. The second method, proposed by Barrenscheen and Dreguss and by Simon and Neuberg, depends on the precipitation of pyruvic acid and methyl glyoxal as the 2, 4-dinitrophenyl-hydrazones which are then estimated colorimetrically. Both these tests are delicate, but as has been intimated, they depend on the accumulation of pyruvic acid and methyl glyoxal and therefore determine only indirectly vitamin $B_1$ deficiency. Furthermore, they are not absolutely specific since acetone combines with bisulfite in the same manner. Finally, the accumulation of pyruvic acid in the blood occurs as a relatively late manifestation of $B_1$ deficiency, thereby restricting the value of the test for early detection.

The method proposed by Peters and his coworkers, utilizing the so-called catatorulin effect, depends on the phenomenon of increased oxygen uptake of polyneuritic pigeon brain tissue *in*

*vitro* in a pyruvate substrate after the addition of vitamin $B_1$. The Warburg respiration apparatus is used. The method has a similar specificity as the other methods previously enumerated for determining accumulation of intermediary products of carbohydrate metabolism (pyruvic acid). The threshold of reaction is 0.1γ crystalline $B_1$, and is applicable only to relatively pure preparations of the vitamin.

The mold growth method of Schopfer has been applied by Meiklejohn to the determination of $B_1$ in the blood. The author, claiming for it an accuracy of approximately ± 5 per cent, states that other growth-promoting substances do not interfere with this test. The mold used is *phycomyces Blakesleeanus*. Since intermediates of vitamin $B_1$ are as effective in promoting growth of this mold as the vitamin itself, it is believed that the mold is capable of synthesizing the vitamin from its precursors. This method is applicable to assays of blood and appears reliable and specific, but only one year has elapsed since its introduction and few studies are as yet available on cases of clinical deficiency.

The yeast fermentation method, proposed by Shultz, Atkin, and Frey, measures the amount of gas liberated during fermentation as an index of the $B_1$ content of the material to be tested. The accuracy of the method, according to the authors, is ± 15 per cent; it is apparently both delicate and specific. Accurate appraisal must await further work.

Only those chemical methods which depend on colorimetric reactions and which might be applied to blood determinations, will be reviewed. They comprise the thiochrome method of Jansen and the adaptations by Kinnersley and Peters and of Prebluda and McCollum of the relatively nonspecific diazo reactions.

The thiochrome test of Jansen depends on the fact that when thiamin chloride is oxidized (dehydrogenated), it loses two hydrogen atoms to form thiochrome, a relatively stable compound, which yields a blue fluorescence in ultraviolet light (Fig. 2). The method consists essentially of oxidizing thiamin chloride or the eluate of an acid-clay adsorbate of $B_1$ or of a Fuller's earth adsorbate of urine dissolved in methyl alcohol, the oxidizing agent being potassium ferricyanide in an alkaline solution. The thio-

chrome which forms is extracted with isobutyl alcohol and the intensity of blue color which develops in the presence of ultraviolet light is determined with the eye directly or with the aid of physical apparatus such as the camera obscura or a photoelectric photometer standardized for thiochrome. The method with certain minor modifications has been used by a number of workers, including Jansen, Karrer and Kubli, Goudsmit and Westenbrink, Widenbauer, Huhn and Becker, and Neuweiler, for assaying the vitamin $B_1$ content of food and urine, the consensus amongst these authors being that the range of error of the method is in the neighborhood of $\pm$ 20 per cent.

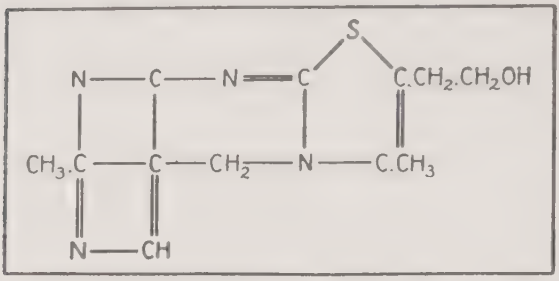


**Fig. 2. Structural formula of thiochrome.**

Unfortunately this method is not entirely specific for vitamin $B_1$. It has been shown that yellow fluorescent flavins and probably natural food substances other than $B_1$ yield fluorescent compounds when oxidized. Furthermore, Williams believes that the quantitative conversion of thiamin to thiochrome by the methods thus far suggested has not yet been achieved. Finally, the method is impaired when applied to urine since a number of workers have shown that urinary pigments, especially urochrome, interfere because they seem to inhibit the formation of thiochrome. However, this method is sufficiently accurate to demonstrate that urinary excretion of $B_1$ varies directly with the $B_1$ content of the diet.

Kinnersley and Peters have used the Pauly reaction for the determination of vitamin $B_1$. This depends on the formation of a characteristic pink or red color with diazotized sulfanilic acid in alkaline solution. The color is then compared visually with that developed by standard solutions of the vitamin similarly treated. Use of a colorimeter has been found unsatisfactory because of the faintness of the color and because of interference by a yellow color due to impurities. The method has been used

chiefly for assaying the $B_1$ content of foods and is not applicable to urine, according to Widenbauer and his coworkers, because of an interfering yellow-brown color which always developed when urine was subjected to the above procedure. They believed that possibly they were unsuccessful because of the rapid destruction of the minute amounts of $B_1$ in urine in the presence of alkali. This color reaction is not specific since a similar color develops from diazotized sulfanilic acid with ergothionine, bile acids, and other thiazoles.

Prebluda and McCollum have found that derivatives of aniline or the naphthylamines, after being treated with nitrous acid (diazotized), produce characteristic colored solutions when allowed to react with vitamin $B_1$ under certain unstated conditions. They have stated that either p-aminoacetanilide or p-aminoacetophenone is suitable for this purpose. These reagents reacted with the vitamin to form purple-red compounds which crystallized rapidly, were stable and highly insoluble in the aqueous medium in which they were formed. These compounds could be extracted with suitable solvents (acetone or isobutyl alcohol) and their concentration accurately determined. The authors state that they were able to determine as little as 1 international unit (3 micrograms of thiamin chloride in 1 cc. of urine). Thus far only two preliminary reports are available in which pure $B_1$ or strong concentrates only were studied. They conclude, "since the color of the reaction product with these reagents is sufficiently sensitive to enable one to detect the presence of one or more international units in 1 cc. of solution, the test is now being made the basis for an accurate quantitative assay of the vitamin in biological materials and foodstuffs."

In conclusion, it seems reasonable to say that the biochemical tests involving the use of color reactions at present available are not reliable for quantitative assay for vitamin $B_1$. This statement is in accord with the views of McCollum, Jansen, and Williams. Color reactions with diazo compounds, with thiochrome and with bismuth in potassium iodide all afford means for the qualitative determination of the presence or absence of $B_1$ in source materials and it is not improbable that, with further refinements

of technic, some or all of the above tests may be made applicable for quantitative assay. For the present, the problem still remains in the domain of the biochemist who, it is hoped, will develop methods for extracting and concentrating the vitamin from its natural sources which will be so quantitatively efficient that a delicate test with rigid specificity for the vitamin may become available. It is not at all unlikely that "with the isolation and synthesis of pure crystalline vitamin $B_1$ and the perfection of microchemical tests by which to analyze the urine for this factor, accurate and time-saving function tests may be devised" since its excretion in the urine varies both with the intake and with the body reserves.

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## DISCUSSION

Dr. Soma Weiss: As to overdosage of vitamin $B_1$, I do not think that this exists. We have given amounts as high as 100 mg. intravenously in a single dose and have been unable to detect any harmful effects whatsoever. As a matter of fact, I should like to state also that with the possible exception of the few questionable cases in the literature of toxic doses of vitamin D in children, there is no such thing as an overdose or a toxic effect in man from high doses of vitamins. Of course, in using very large doses, 10 or 20 times the amounts of vitamin $B_1$ used by us, I do not know what would happen.

As to Dr. Todd's question, these subjects were human beings, and I do not know, therefore, whether the histological changes which occur in the heart are reversible or not. But I do know that the variation in the time element of improvement, as I indicated, can be great. Sometimes considerable improvement occurs within a week; sometimes it takes several weeks.. Whether that has something to do with the fact that an easily reversible process tends to go over to a difficultly reversible process, I do not know. It is possible that in some instances the change may become irreversible. This is the case with some of the other types of avitaminosis, at least as far as histological changes are concerned, and it is possible that it is so in cases of vitamin $B_1$ deficiency.

In the severe cases we have gradually raised our dose and are now using from 20 to 30 mg. three or four times a day. That is entirely empirical, for as yet we have no reliable method for studying saturation of the body. Whether it is necessary to use that amount or not, I do not know, but in view of the fact that these patients have some difficulty with liver function there is a possibility—but not more than a possibility—that they may need high doses.

The combination of pellagra and cardiac and neuritic complications is common. In other words, the combination of pellagra and beriberi is common. The combination of scurvy and beriberi is less common.

# THE RELATION OF NICOTINIC ACID TO PELLAGRA AS EVIDENCED BY THERAPEUTIC STUDIES AND ITS IMPLICATIONS FOR A DIAGNOSTIC TEST

## A PROGRESS REPORT

TOM D. SPIES[1]

GASPAR CASAL, a Spanish physician who in 1735 first described pellagra, shrewdly pointed out that lesions appeared on the dorsum of the hands, feet, and around the neck. He thought that the disease was related to an inadequate diet and that exposure to sunlight might also play a role in its causation, especially noting the skin lesions in this respect.

We now consider pellagra to be a systemic affection involving most, if not all, of the body cells. Although it is a common disease, it is rarely recognized except in its classical forms. However, many investigators are of the opinion that there is a broad range between optimal nutrition and the development of the diagnostic manifestations of pellagra. Recently we have been concerned with detecting the very early clinical manifestations and studying the subclinical form of the disease.

In giving the following progress report we shall make no attempt to discuss the work done by others. Throughout the study, our efforts have been directed toward the development and application of methods by which an earlier diagnosis could be made. These attempts can be divided into those concerned with: (1) Observation of the early symptoms of induced pellagra; (2) laboratory determinations directed towards developing early objective tests; and (3) observations on the effect of pure chemical substances on the various manifestations of clinical and subclinical pellagra.

### STUDIES OF EARLY SYMPTOMS OF INDUCED PELLAGRA

These symptoms can best be described as arising from three organ systems, although such a description is necessarily arbi-

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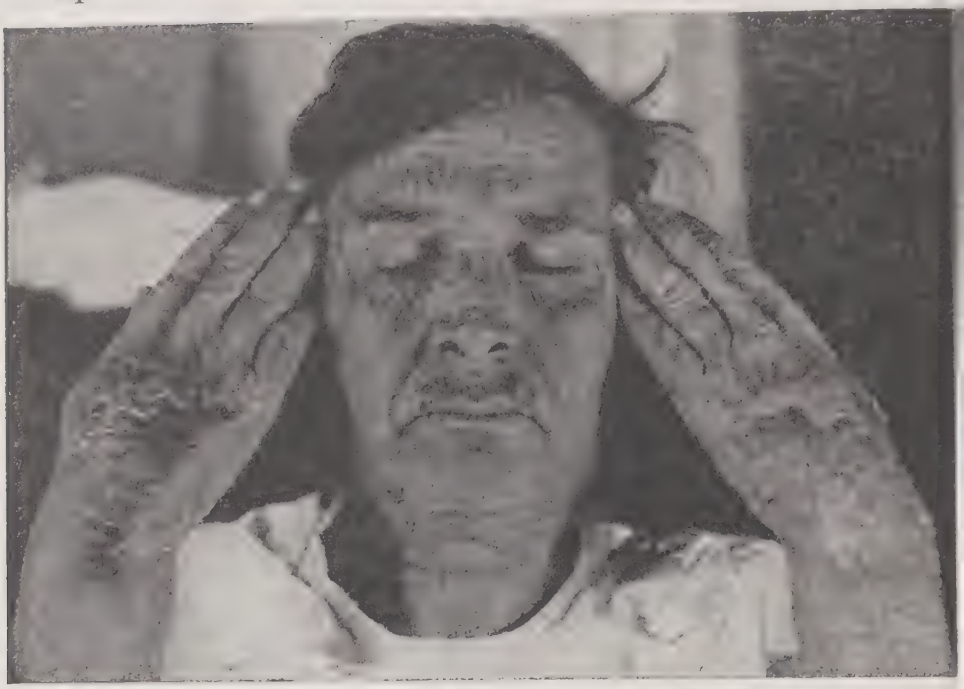

Fig. 1. Dermatitis of the hands, face, and lower lip. Note the bilateral symmetry of the lesions and the swelling of the fingers. Desquamation is apparent on the hands.

trary as all three systems are not always involved, nor are they affected in any regular order.

*The Skin.* The dermal lesions of pellagra may appear on any part of the body, but are most often found over sites of irritation such as the hands, wrists, elbows, face, neck, under the breasts, knees, feet, and in the perineal region (Fig. 1). The areas of dermatitis are in almost every instance bilaterally and symmetrically distributed and are separated from the healthy skin by a sharp line of demarcation (Fig. 2). In the early stages examination usually reveals an erythematous area which closely resembles sunburn and is often accompanied by severe burning and itching (Fig. 3 and 4). As the disease progresses, the area becomes swollen, tense, and more reddened in color (Fig. 5). In some cases vesicles and bullae form, and eventually rupture.

*Alimentary Tract.* Characteristic glossitis and stomatitis usually appear early in the course of the disease. In the early stages of pellagrous glossitis, the tip and lateral margins of the tongue are reddened and swollen. As the disease progresses, the swelling

Fig. 2. Mild dry type of pellagrous dermatitis. The sharp line of demarcation at the wrist and the dry, fissured skin are outstanding.

increases and the reddening becomes more intense. Deeply-penetrating ulcers may appear along the sides and tip, but are rarely seen on the surface, this area frequently being covered with a thick gray membrane filled with debris and Vincent's organisms. The tongue is usually hypoaesthetic, but it may be hypersensitive. The buccal membranes, the mucotaneous surface of the lip, gums, and the palate may be affected likewise. The course of these oral lesions is similar to that of the glossitis. A burning sensation of the tongue and of the mucous membranes of the pharynx, esophagus, and stomach is frequently present and is sometimes aggravat-

Fig. 3. Early stage of pellagrous dermatitis. Note the bilateral symmetry of the lesions and the swelling of the fingers.

Fig. 4. Very early stage of pellagrous dermatitis. Bilateral erythema and slight swelling of the fingers of the right hand.

ed by hot or acid foods. Anorexia, ptyalism, nausea and vomiting may occur early but are usually advanced symptoms of the disease. About 60 per cent of pellagrins have achylia gastrica. The bowels in the majority of mild cases act naturally or are constipated. Severe persistent diarrhea tends to appear in the more acute cases. Abdominal distention, discomfort, and pain may be present at any time during the course of the disease.

*The Nervous System.* Nervous symptoms are common but at the onset are often vague and ill-defined. The person is often subject to periods of depression and apprehension and may complain of increased irritability, headaches, dizziness, insomnia, muscular weakness, impaired memory, and bilateral burning of the extremities or other parts of the body. In the early stages tendon reflexes are usually increased. "Numbness" of the extremities, particularly the legs, is a common finding. If the patient is not treated, insanity often develops.

### LABORATORY STUDIES

After observing the early clinical symptoms of pellagra over a considerable period of time, it seemed desirable that we devise a physical or chemical method which could be used as an aid

to early diagnosis. Beckh, Ellinger, and I decided that since there was controversy about photosensitization and the skin lesions, we would try to establish whether or not pellagrins had increased amounts of photosensitizing agents in the urine. It soon became apparent that urine from pellagrins often contained an ether-soluble porphyrin, but none of the ether-insoluble porphyrins. Accordingly, the following method was devised so that it could be applied with little equipment but still give sufficiently accurate results even with small amounts of urine:

**Fig. 5. Photograph of the same patient seen in Figure 3 taken two months later. No treatment was given during the two months' period. The dermatitis now involves the forearms as well as the hands. Swelling of the hands has passed the maximum, but is still greater than that seen in the previous photograph.§**

A measured portion, e.g., 10 cc., of urine, preferably of a 24-hour specimen, is put into a separatory funnel and acidified with glacial acetic acid (about 0.2 cc.) to a pH of 4.0. Fifteen to 20 cc. of ether is added and the mixture is shaken for 2 minutes to extract the porphyrin, or porphyrin-like substances. The lower aqueous layer is separated out and the ether portion is washed twice with from 10 to 15 cc. of distilled water. To the ether extract is added 3 cc. of 25 per cent HCl. The mixture

§ Figures 1, 2, 3, 4, and 5 are reproduced through the courtesy of the J. B. Lippincott Company, Philadelphia, Pennsylvania.

is shaken and then is transferred to a test tube in which the acid and ether layers are allowed to separate. The hydrochloric acid portion is examined for porphyrin content. In a positive specimen the acid layer is definitely colored from pink to purple; in a negative specimen the layer is colorless or slightly colored.

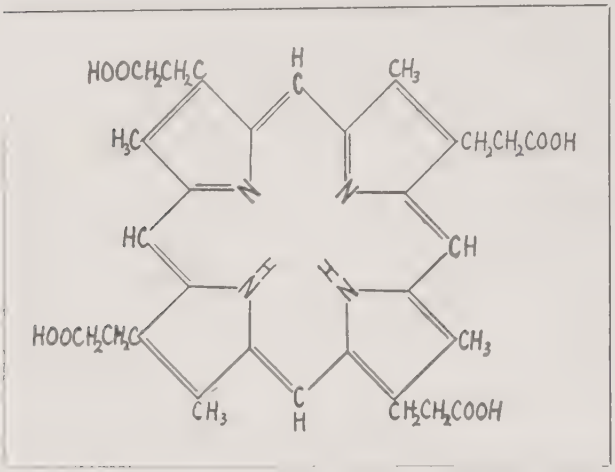


Fig. 6. Structural formula of coproporphyrin, type I.

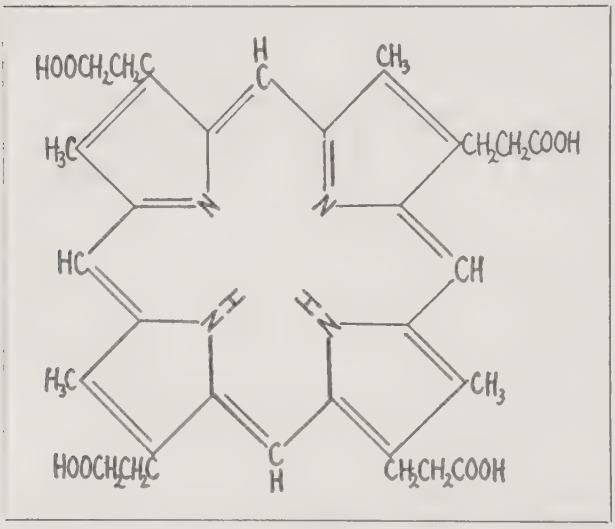


Fig. 7. Structural formula of coproporphyrin, type III.

When convenient it is desirable to confirm this test by concentrating the porphyrin content in the urine. Spectroscopic examination of the 25 per cent HCl extract may then be made. The spectrum absorption bands concerned with the porphyrin in the urine of pellagrins are characteristic of type I and type III coproporphyrin (Fig. 6 and 7). In making our determinations we have been aided greatly by specimens of pure coproporphyrin obtained through the courtesy of Dr. Hans Fischer and Dr. Paul Rothemund. Dr. C. J. Watson has suggested that mesobiliviolin might give a similar color under the conditions of this test. He has supplied us with mesobiliviolin and we have found that it does appear

similar. We have not yet determined whether mesobiliviolin occurs in the urine of pellagrins. Still other substances probably give this same coloration. Isolation of the pure porphyrin crystals entails elaborate and painstaking preparation and requires a still larger quantity of urine. This step is necessary, however, if one wishes to determine whether the coproporphyrin is type I or III.

Under the conditions of our study we have observed that there is an increased amount of porphyrin in the urine of pellagrins and that the porphyrin content of the urine returns to normal after administration of yeast, liver extract, wheat germ, or nicotinic acid. A preliminary study of 4 cases indicates that the urobilinogen in the urine was lowered after treatment with large amounts of nicotinic acid. Urobilinogen excretion is extremely variable and study of only 4 cases has little, if any, significance. This study is still in progress.

### THE DETERMINATION OF NICOTINIC ACID, NICOTINAMIDE, AND OTHER PYRIDINE-LIKE SUBSTANCES IN HUMAN URINE

Following the discovery by Elvehjem and his associates that nicotinic acid would cure black tongue in dogs and the subsequent work of Spies, Cooper, and Blankenhorn demonstrating its curative value in human pellagra, we realized the need for a test which would identify nicotinic acid and determine the amount in the urine (Fig. 8). Such a method is herewith described.

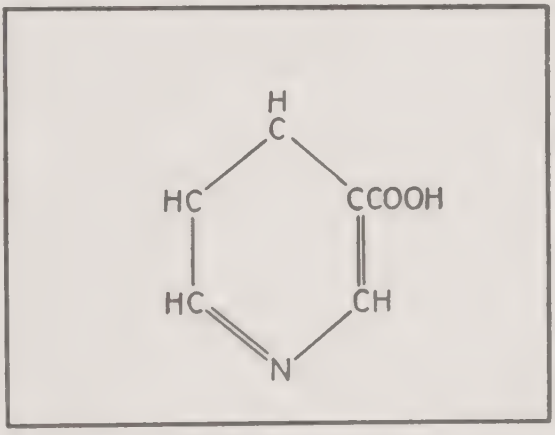


Fig. 8. Structural formula of nicotinic acid.

Fifteen cc. of urine are decolorized by boiling with 0.1—0.3 gm. of charcoal. The solution when filtered should be clear and colorless. Three cc. samples are then measured into 30 cc. beakers and are evaporated just to

dryness in an oven at 105° C. When dry, the beakers are removed and 1 cc. of an alcoholic solution of 2,4-dinitrochlorobenzene (1 gm. in 100 cc. of alcohol) is added. Ten milligrams of 2,4-dinitrochlorobenzene is sufficient for about 5 milligrams of pyridine substances. The samples are again evaporated to dryness; this time a water-bath can be used in place of the oven, since the reagent will unite with the pyridine ring at the temperature of the bath. Samples which are browned by overheating should be discarded. The beakers are cooled to room temperature and 10 cc. of a clear, cold (10° C.) solution of 0.1 per cent sodium hydroxide in 95 per cent alcohol are added and the residue stirred from the bottom. The intensity of the red-orange color which develops is a measure of the nicotinic acid derivatives which are present.

At times, the urine residue seems to retain some of the color; hence an alternate method has been tried. Duplicate samples were carried through the same procedure until the alkali was added. At this point, 13 cc. of cold 0.1 per cent alcoholic sodium hydroxide were added. When the color had developed, 2 cc. of water were added to dissolve the residue and free the color. The water, however, hastened the fading-time, so this method has not been used.

Standard solutions (0.1 - 0.5 mg.) of nicotinic acid or nicotinamide, similarly treated with 2,4-dinitrochlorobenzene and alkali, will develop purple-red and burgundy-red colors, respectively. A blank determination of comparable quantities of the reagent with alkali should always be performed when fresh solutions are prepared, as too concentrated alkali produces a decided yellow color with the 2,4-dinitrochlorobenzene. Such a blank is convenient also, when matching colors.

Since the colors fade rapidly, the quantitative reading must be made at once while the solution is cold. The depth of color is easily determined by a photelometer, using a green filter. The photelometer should be calibrated and curves obtained for standard solutions of nicotinic acid and nicotinamide (within the limits of 0.1 - 0.5 mg.). If the solutions are clear before reading, they must be quickly filtered while cold. From the photelometer reading, the amount of nicotinic acid or nicotinamide in unknown solutions can be read from the respective curves, provided only the acid or amide is present.

Unfortunately the colors of these are not the same, so two curves have to be used. Furthermore, in the urine it is probable that both substances are present, so the color formed is a composite of two or more reds. If a photoelectric spectrophotometer is available, it may be possible to determine the amount of nicotinic acid and nicotinamide occurring simultaneously. However, we have found the percentage of absorption a reliable index of the amount of pellagra-preventive substance in human urine. If it is negative, no color-producing substances are present in the urine. This is the case in the pellagrins so far examined. Also, there is very little color developed by normal individuals on a strictly controlled "pellagra-producing" diet.

This test is not specific, however, for nicotinic acid or nicotinamide, for a similar reaction with pyridine is well known. There are, however, only two other pyridine compounds at present known to occur in normal urine, namely trigonellin and methylpyridinium ammonium hydrate, and there is reason to believe that these substances will not develop color under the conditions of this reaction, so a positive reaction is fairly certain to be produced either by nicotinic acid or its amide.

### RESPONSE OF PELLAGRINS TO NICOTINIC ACID

After Elvehjem reported that canine black tongue was cured by the use of nicotinic acid, we decided to study the effect of this substance on human beings. As it had never been administered to persons, we made a number of preliminary tests before we reached the conclusion that it could be given safely in adequate amounts by mouth or parenteral injection. Some of the pellagrins received food and water during the time of study; others received only parenterally injected glucose and others received special control diets low in anti-pellagric factor.

### SUMMARY AND CONCLUSIONS

From observations which have been reported and from studies which are still in progress, it seems justifiable to draw the following conclusions:

1. Nicotinic acid is a valuable therapeutic agent in the treatment of pellagra as evidenced by the fact that 77 cases of severe adult pellagra have been treated successfully.

2. Eighteen selected mental patients, all of whom were disoriented as to time, place, and person, had a return to sanity within 24 to 48 hours following the administration of nicotinic acid. Insanity in every instance was due to pellagra.

3. One hundred and ninety-nine pellagrins who have had from one to two recurrences of the disease each year have not had recurrences so long as they have continued to take nicotinic acid. However, some of these persons have developed mild beriberi, which was relieved by synthetic vitamin $B_1$ (thiamin hydrochloride).

4. Studies of children from "pellagra families" have shown that many of them had signs of clinical pellagra. Following the administration of nicotinic acid or some of its compounds they have had spectacular improvement. Others without the usual diagnostic evidences, but in whom we suspected subclinical pellagra, were also benefited.

5. The methods devised for determining porphyrin and nicotinic acid excretion in the urine seem to have value as confirmatory tests for pellagrous and prepellagrous states. Pellagrins in relapse tend to excrete less nicotinic acid than normal people.

6. The precise effect of nicotinic acid on the cells of the body is not known. The studies presented here suggest that its lack leads to cellular changes in the alimentary tract, skin, nervous system, and other parts of the body.

7. These studies aid in confirming the opinion that pellagra is a reaction of the body to a lack of essential nutrient substances, rather than just a dermatitis.

8. From careful interpretation of the history and the physical findings with particular reference to early symptoms and from determinations of porphyrin content and nicotinic acid in the urine, we find we can recognize pellagra much earlier in the course of the disease than we had previously recognized it.

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# TITRATION OF PLASMA ASCORBIC ACID AS A TEST FOR LATENT AVITAMINOSIS C

CHESTER J. FARMER AND ARTHUR F. ABT[1]

THE approaches to the chemical estimation of vitamin C[2] have been chiefly based upon the color changes resulting from reduction of one of the three substances: phosphotungstic acid, methylene blue or 2,6 dichlorophenol-indophenol. Use of the latter dye, which Tillmans first adapted to vitamin C determination, has been most widely applied. *l*-Ascorbic acid, which is the lactone of the 2, 3 dienol of *l*-gulonic acid, is the reduced member of a reversible oxidation—reduction system, and when treated with such an oxidant as the indophenol dye, changes into the oxidized form, dehydroascorbic acid. Due to the reversibility of this reaction, the latter product may be reduced to *l*-ascorbic acid upon treatment with hydrogen sulfide. These two ascorbic acid compounds are the only known antiscorbutic substances occurring in nature. Although both are biologically active, only the reduced form, *l*-ascorbic acid, will reduce the indophenol dye and thus permit titration; hence the indophenol method determines the amount of reduced ascorbic acid. Since only this form occurs in the animal body, if precaution is taken against extraneous oxidation, the estimation of the reduced ascorbic acid in bodily tissues becomes an accurate measure of their vitamin C content.

After working out a macro-method for blood which required a 5 cc. sample from the vein, we abandoned it in favor of the micro-method which, in addition to furnishing equally reliable results, may be used more conveniently with children. Three-tenths of a cubic centimeter of blood, from the ear or finger, is collected in a small glass phial (Fig. 1-*a*), containing a little potassium oxa-

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[2] Vitamin C, ascorbic acid, and cevitamic acid, are used here as synonymous terms referring principally to the antiscorbutic substance, *l*-ascorbic acid. Cebione is the name of a proprietary product containing the same substance.

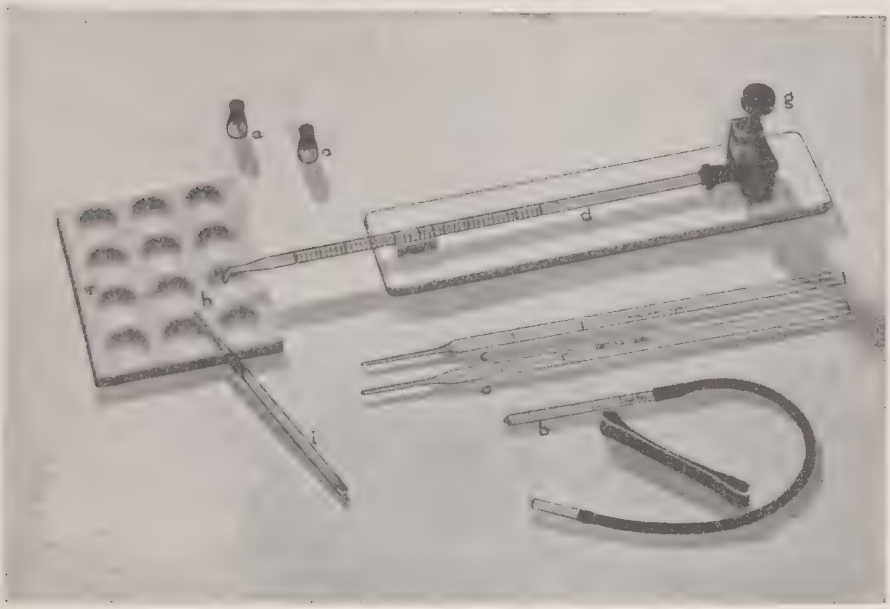


**Fig. 1. Apparatus for micro-method. Blood plasma ascorbic acid.**

late. This amount of blood fills the phial so that when stoppered very little air space remains. If the pipette method is preferred in taking the sample, a collection tube *(b)* with oxalate in its bore may be used for drawing up the blood, after which it is sealed by placing a rubber band around its open ends. In either instance the sample is centrifuged to obtain plasma. For deproteinization the plasma is transferred from the glass phial, or the tube, to a centrifuge tube by a capillary pipette *(c)*.

After having tried several deproteinizing agents we have found that the action of 5 per cent metaphosphoric acid on the plasma of oxalated blood is the most satisfactory. Ten per cent trichloracetic acid gave no sharp end-point. We tried, at first, drawing blood into sodium tungstate which prevents coagulation; addition of water and sulphuric acid then causes deproteinization. But this procedure incurred a loss of ascorbic acid; attempts to rectify it by washing the precipitate gave recovery values that were 3 or 4 times the amount of added ascorbic acid. Thus metaphosphoric acid, which introduces no appreciable loss, became the deproteinizing agent of choice.

The reduced cevitamic acid content in the protein-free fluid is

determined by titration with a standardized solution of the dyestuff (2,6 dichlorophenol-indophenol). We always standardize our dye by titration against crystalline ascorbic acid, and in turn the latter is titrated against a standard iodine. Samples of ascorbic acid have been encountered which showed from 97.8 to 99.8 per cent of the theoretical amount. Consequently it is necessary for very accurate work to check the strength of the crystalline ascorbic acid by iodometric titration. The dye tablets, placed on the market, should then be checked against the ascorbic acid which has been standardized by iodine, since different lots may vary in dye content, some giving values which are at least 6 per cent too low. This influences the end results. If the dye tablets are too low, then the calculated blood values are bound to be high.

Titration is conducted with a microburette *(d)* which is a capillary tube of fairly uniform bore, with mercury as the medium of operation. The burette is filled by placing it into the end of an inclined test tube containing the dye, and then compressing the rubber sac containing the mercury. All of the air must be expelled in filling the sac else the mercury column will not follow directly the manipulation of the knurled screw *(g)*. One drop of mercury is expressed into the test tube; then the knurled head is turned in the opposite direction so that the mercury within the tube reascends with the dye following it.

Two-tenths cc. of the protein-free filtrate is now placed in a spot-tile *(h)*; and 0.2 cc. of 2.5 per cent metaphosphoric acid solution in an adjacent hollow, this latter corresponding to the strength of the acid in the deproteinized fluid. There are two ways of conducting the titration. An actual contact may be made between the 0.2 cc. of fluid in the tile and the curved end of the capillary burette, or a stirring rod *(i)* may be used in taking the smallest portion of a drop from the end of the burette and adding it to the material in the tile depression. The first visible change in color, that is, the first visible pink, must be taken as the end-point; it is quite delicate.

In determining the end-point the first thing to note is the diffusion of the dye into the filtrate solution that is being titrated.

It is the speed with which the diffusion line fades that is the index of an approaching end-point. At the end-point, the diffusion line will gradually disappear and the fluid take on a uniform faint pink color. After the end-point is attained in the plasma sample, and the reading of the burette recorded, the blank is immediately titrated bringing it up to the same intensity of color as that of the plasma sample. In this manner individual titrations are self-compensating.

The titration, whenever possible, should be made in daylight. Artificial light is unsatisfactory, and should not be attempted unless the individual is experienced with the method. A recent communication by Wilson (1938) describes an aid in obtaining uniform end-points.

On the microburette 0.1 cc. is divided into 50 parts so that each division is equal to 0.002 cc. Mid-points between the divisions on the scale may be easily estimated. The blank, which may be clearly discerned, is usually around two-thousandths of a cubic centimeter.

With these details in mind the method may be summarized as follows:

PRICK THE FINGER, and collect 6-8 drops (0.3 - 0.4 cc.) blood in phial, which contains a little potassium oxalate. Stir with a toothpick, stopper, and centrifuge.

DEPROTEINIZE: Pipette 0.1 cc. plasma plus 0.1 cc. water plus 0.2 cc. fresh 5 per cent metaphosphoric acid solution into a 15 cc. conical centrifuge tube. Mix thoroughly. Centrifuge down the coagulated protein.

TITRATION with sodium 2,6 dichlorophenol-indophenol. Fill the microburette from dye solution placed in a clean test tube held nearly horizontally when slipped over the curved end of the burette. Turn the screw to the right until a small drop of mercury is expelled into the dye solution; then fill to desired point by turning screw in reverse direction.

Place 0.2 cc. of 2.5 per cent metaphosphoric acid in a depression of the titration tile. Into an adjacent tile depression pipette 0.2 cc. of the sample of deproteinized plasma, the ascorbic acid content of which is to be estimated. Read the microburette. Now titrate the deproteinized plasma until the first discernible

trace of color (faint pink) is obtained, in comparison with the metaphosphoric acid solution in the adjacent depression. After taking the reading from the burette, the metaphosphoric acid solution is titrated until the color matches that of the plasma (which has just been titrated). The burette reading is again taken, and this value is subtracted from the reading of the plasma. Make plasma titrations in duplicate.

For the preparation of the standard dye solution, see below.

CALCULATION: (Cc. dye − cc. blank) x S x 2,000 = milligrams cevitamic acid (reduced form) per 100 cc. blood plasma.

S = mg. ascorbic acid equivalent to 1 cc. dye.

EXAMPLE: Dye used plasma titration = 0.026 cc.

Blank = 0.002 cc.

S = 0.02

Therefore, (0.026 − 0.002) x 0.02 x 2,000 = 0.96 mg. per 100 cc. plasma.

For a standard dye solution for clinical work, place 1 tablet of sodium 2,6 dichlorophenol-indophenol in a 50 cc. volumetric flask. Dissolve in distilled water, finally making up to 50 cc. volume. Mix thoroughly. One cc. dye solution is equivalent to 0.02 mg. ascorbic acid. The tablets we use may be obtained from Hoffman-LaRoche, Inc., Nutley, N. J., or from many jobbers. E. H. Sargent and Co., 155 E. Superior St., Chicago, makes the apparatus for us and can supply the dye tablets. For standardization of dye when research is in progress, we recommend titration against crystalline vitamin C, particularly that which is placed on the market in 0.1 gm. vacuum sealed ampoules. This is checked against iodine.

Table 1. Recovery values for ascorbic acid in 2 per cent metaphosphoric acid solution.

| Ascorbic Acid Solution Taken Ml. | Blank Ml. Dye | Dye Ml. | Ascorbic Acid Recovered Mg. |
|---|---|---|---|
| .2 | .005 | .043 | .00106 |
| .2 | .005 | .042 | .00103 |
| .2 | .005 | .043 | .00106 |
| .2 | .005 | .043 | .00106 |
| .2 | .005 | .044 | .00109 |
| .2 | .005 | .043 | .00106 |
| Theoretical Value | | | .00110 |

Table 1 demonstrates the consistent titration values which we obtained with the method, indicating its reliability. It may be seen that with ascorbic acid dissolved in 2 per cent metaphosphoric acid solution, practically theoretical results were obtained. In order to ascertain the stability of the deproteinized sample and the limits of time within which the test should be conducted, as revealed by recovery values of a sequential series, we titrated the blood from the same person after it had been held for various intervals. When the plasma was deproteinized immediately and placed in the refrigerator at a temperature not exceeding 4°, it yielded approximately the same values even after standing for 24 hours (Table 2). Table 3 presents comparative values obtained by the macro and micro-methods. The micro-method gave values that were for the most part slightly higher than those obtained by the macro-method.

Table 2. Stability of ascorbic acid in deproteinized blood plasma. Millimeters of dye reduced by 0.2 ml. metaphosphoric acid deproteinized plasma.

| Titrated Immediately* Ml. Dye | Titrated After Twenty-Four Hours† Ml. Dye |
|---|---|
| .010 | .010 |
| .012 | .012 |
| .010 | .010 |
| .010 | .010 |
| .014 | .012 |
| .010 | .010 |
| .009 | .010 |

*Samples taken from same person, at thirty minute intervals.
†After standing in refrigerator at 4° C. for twenty-four hours.

Table 3. Comparative values of macro and micro-methods.

| Plasma Sample | Ascorbic Acid | |
|---|---|---|
| | 2 Ml. Portion (Macro) Mg. Per Cent | 0.2 Ml. Portion (Micro) Mg. Per Cent |
| 1 | 1.75 | 1.82 |
| 2 | 0.70 | 0.84 |
| 3 | 0.98 | 1.12 |
| 4 | 0.54 | 0.53 |
| 5 | 1.35 | 1.15 |
| 6 | 0.86 | 0.76 |
| 7 | 0.63 | 0.77 |

In order to ascertain normal values for the concentration of reduced ascorbic acid in the plasma of infants and children, determinations were made on a group from the age of 1 week to 13 years, all of whom had received a diet adequate in vitamin C. The values ranged from 0.75 to 2.42 mg. per cent (Table 4 and Fig. 2). In infants and children with a low vitamin C dietary,

Table 4. Normal values obtained on patients in Pediatrics Clinic of Northwestern Medical School.

| Subject | Age | Plasma Reduced Ascorbic Acid Mg. Per Cent | Capillary Resistance of Skin Mm. of Hg. | Comments | R.B.C. in Millions | Hemoglo | |
|---|---|---|---|---|---|---|---|
| | | | | | | Gm. | P Ce |
| O | 3 da. | 1.13 | 500 | No Jaundice | | | |
| T | 3 da. | 1.61 | 600 | No Jaundice | | | |
| K | 4 da. | 0.90 | 600 | No Jaundice | | | |
| D | 5 da. | 1.08 | 500 | No Jaundice | | | |
| C | 6 da. | 0.752 | 500 | Very Slight Jaundice | | | |
| E | 6 da. | 1.24 | 600 | No Jaundice | | | |
| N. K. (c) | 7 wk. | 1.180 | 300 | Breast Plus O.J.* Daily | 4.520 | 13.4 | 9 |
| W. E. | 2 mo. | 0.975 | 500 | Formula Plus O.J. Daily | | | |
| D. V. | 2 mo. | 1.29 | 400 | Breast Plus O.J. Daily | 3.650 | 11.8 | 8 |
| F. P. | 2 mo. | 1.237 | 350 | Breast Plus O.J. Daily | 3.365 | 10.6 | 7 |
| C. M. | 2½ mo. | 1.232 | 450 | Whole Milk Formula. Very Small Amount O.J. Daily | | | |
| C. B. | 3 mo. | 2.416 | 400 | Breast Plus O.J. Daily | 3.570 | 10.0 | 7 |
| D. H. | 3 mo. | 1.007 | 250 | Formula Plus O.J. Daily | 3.340 | 10.0 | 7 |
| H. L. | 4 mo. | 1.01 | | Breast Plus O.J. Daily | 3.990 | 9.6 | 6 |
| L. W. (c) | 5 mo. | 1.166 | 550 | Breast Plus O.J. Daily | 5.710 | 11.8 | 8 |
| J. S. | 6 mo. | 1.85 | 350 | Breast Plus O.J. Daily | 4.570 | 11.3 | 7 |
| D. E. | 6½ mo. | 1.234 | 450 | Formula Plus O.J. Daily | 4.645 | 12.4 | 8 |
| J. G. | 7 mo. | 1.155 | 450 | Breast Plus O.J. Daily | 4.920 | 12.2 | 8 |
| J. W. (c) | 8 mo. | 0.832 | 300 | Formula Plus O.J. Daily | 4.250 | 9.2 | 6 |
| R. J. | 9½ mo. | 0.968 | 550 | Breast Plus O.J. Daily | 4.380 | 10.8 | 7 |
| F. J. (c) | 11 mo. | 1.568 | 250 | Breast Plus O.J. Daily | 4.840 | 11.2 | 7 |
| D. M. | 1 yr. | 1.168 | 550 | Cow's Milk, Vegetables, O.J. Daily | 5.150 | 8.4 | 5 |
| D. F. | 16 mo. | 1.129 | | | | | |
| C. W. (c) | 17 mo. | 1.771 | | Whole Milk, 4 Oranges Daily | | | |
| D. K. | 19 mo. | 0.909 | 250 | Vegetables, Fruit, Oranges Daily | 5.250 | 9.8 | 6 |
| A. K. | 2 yr. | 1.236 | 250 | Qt. Milk, O.J. Daily | 5.300 | 10.2 | 7 |
| L. D. | 2 yr. | 1.560 | 350 | O.J. Daily. Cooked Vegetables and Fruits | 4.630 | 11.2 | 7 |
| W. H. | 2 yr. | 2.232 | 300 | O.J. Every Other Day | | | |
| J. G. (c) | 2 yr. | 1.298 | 250 | Few Fruits or Vegetables | 4.635 | 12.0 | 8 |
| G. S. | 2½ yr. | 1.61 | 450 | O.J. Daily. Raw Vegetables | 4.070 | 11.8 | 8 |
| M. S. | 2½ yr. | 0.890 | 300 | O.J. Daily. Raw Fruit and Vegetables | | | |
| T. J. | 2¾ yr. | 1.644 | 100 | Oranges and Apples Daily | 4.800 | 11.4 | 7 |
| M. O. | 3 yr. | 0.819 | 450 | | 4.710 | 12.4 | 8 |
| J. D. | 3 yr. | 1.344 | 200 | O.J. Daily, Much Milk, Cooked Vegetables | 4.350 | 12.6 | 8 |
| E. L. | 3 yr. | 1.101 | 450 | | 4.900 | 11.8 | 8 |
| R. B. | 3½ yr. | 2.033 | 400 | High Carbohydrate Diet | 4.670 | 11.6 | 7 |
| B. K. | 4 yr. | 1.812 | 250 | Eats All Fruits and Vegetables | 4.260 | 11.6 | 7 |
| T. B. | 4 yr. | 0.802 | 500 | High Carbohydrate Diet | 4.980 | 10.2 | 7 |
| M. J. | 5 yr. | 1.220 | 350 | O.J. Daily | 4.770 | 13.0 | 8 |
| D. B. (c) | 5 yr. | 0.887 | 450 | Cooked Vegetables | 4.790 | 12.0 | 8 |
| R. B. | 5 yr. | 0.821 | 250 | Oranges Daily | 5.140 | 12.8 | 8 |
| W. M. | 6 yr. | 0.859 | 300 | Three to Four Oranges per Week. Apples Frequently | 4.720 | 12.8 | 8 |
| E. D. | 6 yr. | 1.637 | 350 | Apples Frequently | 5.590 | 10.6 | 7 |
| C. P. | 6 yr. | 2.025 | 300 | O.J. Daily, Many Raw Vegetables | 5.140 | 12.2 | 8 |
| M. W. (c) | 7 yr. | 0.802 | | "Green" Vegetables | | | |
| H. H. | 7 yr. | 1.56 | 400 | | 4.450 | 12.2 | 8 |
| W. E. (c) | 7 yr. | 1.388 | 200 | Restricted Diet for Past 2 Weeks Because of Discontinuance of "Relief" | 6.280 | 14.2 | 9 |
| J. J. (c) | 7 yr. | 0.914 | 200 | Asthmatic. Until 4 Yr. Could Take No Citrous Fruits; Can Take Them Now | 5.660 | 12.8 | 8 |
| K. P. | 9 yr. | 1.374 | 125 | Fruits and Vegetable Daily | | | |
| V. Z. | 11 yr. | 0.827 | 400 | | 4.150 | 9.8 | 7 |
| H. C. | 11 yr. | 0.870 | 300 | Fruit Occasionally | 5.200 | 9.8 | 7 |
| C. W. (c) | 11 yr. | 0.870 | 200 | Apples Daily. Oranges Frequently | | | |
| P. H. | 13 yr. | 1.031 | 400 | Oranges Frequently | | | |
| B. M. | 13½ yr. | 0.819 | 300 | No Fruit Since Return From Ridge Farm Week Ago | 5.570 | 11.0 | 7 |

*O.J. = orange juice.
(c) = negro patients.

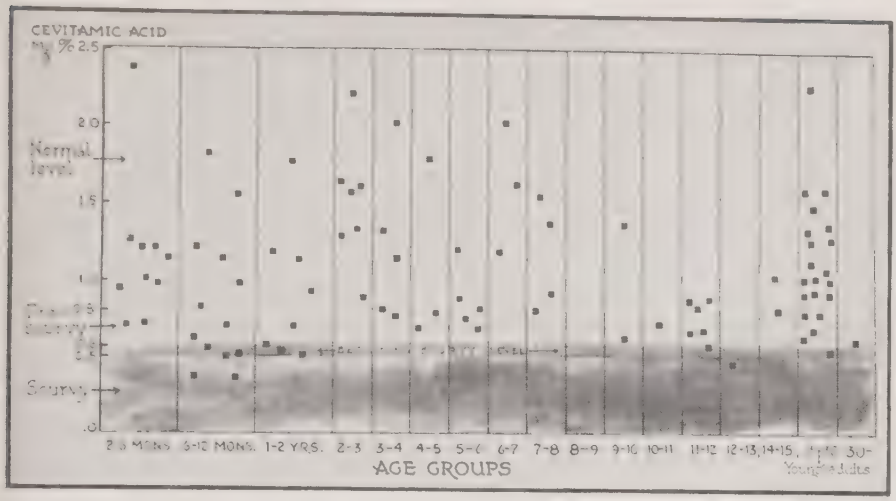


Fig. 2. Normal and abnormal plasma ascorbic acid values.

the values ranged from 0.53 to 0.77 mg. per cent (Table 5). Thus in the group with the lesser dietary intake the values were consistently lower than in the normal group. Those infants with low values may be regarded as having subclinical scurvy.

In our initial series of studies on the application of the micromethod, the reduced ascorbic acid plasma levels in a group of

Table 5. Subnormal values obtained on patients in Pediatrics Clinic of Northwestern Medical School.

| Subject | Age | Plasma Ascorbic Acid Mg. Per Cent | Capillary Resistance of Skin Mm. of Hg. | Comments | R.B.C. in Millions | Hemoglobin | |
|---|---|---|---|---|---|---|---|
| | | | | | | Gm. | Per Cent |
| M. T. | 6 wk. | 0.752 | 450 | Very Little Orange Juice | 4.250 | 10.5 | 72 |
| C. A. | 2 mo. | 0.702 | 500 | Very Little Orange Juice | 3.800 | 10.6 | 76 |
| C. U. | 5 mo. | 0.739 | 500 | Juice ½ Orange Daily | | | |
| M. T. | 6 mo. | 0.675 | 400 | Very Little Orange Juice | 4.510 | 11.6 | 79 |
| W. P. | 8 mo. | 0.513 | 400 | Orange Juice Discontinued Because of Rash | 4.920 | 11.6 | 79 |
| K. L. | 17 mo. | 0.698 | 200 | Very Little Orange Juice | 4.760 | 10.4 | 72 |
| D. W. | 20 mo. | 0.528 | | Very Little Orange Juice | | | |
| P. W. | 3 yr. | 0.769 | 400 | No Orange Juice. Whole Milk | 4.440 | 12.4 | 84 |
| D. E. | 4 yr. | 0.697 | 400 | Orange Juice Daily. Cooked Vegetables | 4.645 | 12.4 | 84 |
| G. B. | 5 yr. | 0.760 | 500 | High Carbohydrate Diet | 4.130 | 10.2 | 74 |
| G. B. | 5 yr. | 0.688 | 250 | Oranges Occasionally | 5.160 | 12.0 | 80 |
| L. H. | 10 yr. | 0.718 | 150 | Bread and Butter Main Diet | 5.510 | 14.2 | 98 |
| G. H. | 11 yr. | 0.688 | | | | | |
| C. B. | 11 yr. | 0.572 | 300 | Oranges Occasionally | 4.870 | 11.2 | 77 |

medical students were determined. The students were then provided with a pint of orange juice daily for a week, at the end of which the levels were again estimated. In this study, the initial blood plasma levels were correlated with the dietary histories. Those students with an initially high blood value of ascorbic acid, reflecting a satisfactory previous intake of vitamin C, showed little or no increase after the week of rather abundant supply of orange juice (Fig. 3). Those with initially low blood values, corresponding to a previously unsatisfactory intake of vitamin C, showed as the result of the orange juice supplement, increases in their blood levels, reaching values nearly equal to those of the initially high group. From these data it is concluded that the reduced ascorbic acid content of the plasma depends upon the amount of vitamin C intake.

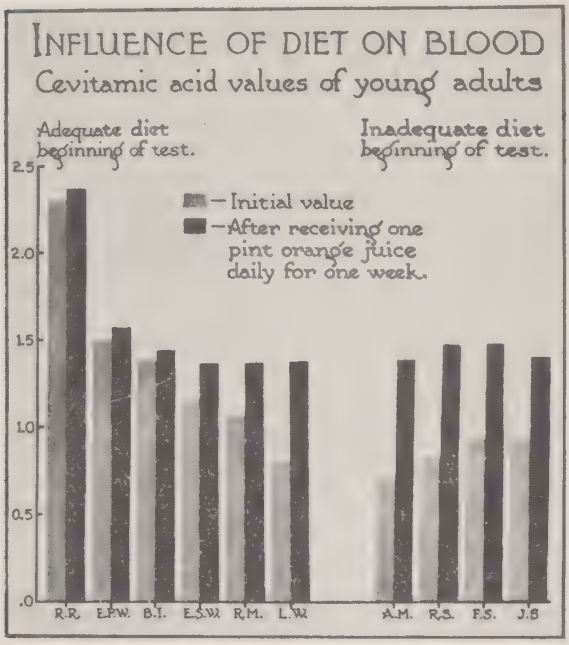


Fig. 3. Diet and plasma ascorbic acid levels.

Similar increased values for reduced ascorbic acid in blood plasma were obtained after administration of crystalline vitamin C to a small miscellaneous group of patients who had subnormal values because of a diet low in the vitamin (Table 6). Their ages ranged from 1 to 32 years, and the dosage of vitamin C was adjusted accordingly, larger amounts being given to the older individuals. In one individual receiving 30 mg. daily for one week, the value increased from 0.5 to 1.7 mg. ascorbic acid per 100 cc. plasma. In another individual with active scurvy, after receiving 30 mg. vitamin C daily for 21 days, the plasma level reached 1.0 mg. per cent. The results obtained with crystalline

vitamin C were similar, on the basis of dosage, to those obtained with a dietary supplement of citrus fruit juice.

Although we believe that a determination of the fasting plasma level can give an accurate index of the state of the body as influenced by previous vitamin C intake, we have employed additional procedures in order to establish the relation of the plasma level to tissue saturation. A determination of reduced ascorbic acid in the urine has been made following the oral administration of a test dose of ascorbic acid. In our experience, neither a single specimen nor one 24-hour sample gives the requisite information concerning a previous vitamin C dietary, or the degree of tissue saturation. When successive test doses were given to individuals low in vitamin C, we frequently obtained no definite increase in urinary output on the second or third day. This is contrary to the experience of Harris and associates. For example, individuals who had not received vitamin C with any degree of regularity but yet showed no clinical manifestations of scurvy, were examined for the initial fasting level in the blood. After the administration of a test

| Subject and Age | Ascorbic Acid Content: Blood Plasma Mg. Per Cent | Ascorbic Acid Content: Urine 24 Hr. Specimen Mg. Per Cent | Comments |
|---|---|---|---|
| E. R. 1 yr. | 0.67 | | No Orange Juice Since Birth |
| | 0.51 | | 19 Days Later Still No Orange Juice |
| | 1.68 | | 30 mg. Cebione Daily for One Week |
| | 1.05 | | 2 Mo. Later, Had Been Having Orange Juice Daily |
| M.H. 9 yr. | 0.67 | 3.42 | No Fruits or Vegetables for Some Time |
| | 1.77 | 5.982 | 50 mg. Cebione Daily for Two Weeks |
| E.H. 11 yr. | 0.67 | 2.52 | No Fruits or Vegetables for Some Time |
| | 1.33 | 13.007 | 50 mg. Cebione Daily for Two Weeks |
| D.W. Adult | 0.67 | | Seldom Eats any Citrous Fruits. Sensitive to Them |
| | 2.01 | | 60 mg. Cebione Daily for One Week |
| E.G. Adult | 0.34 | | Severe Anemia. Had been Living on Diet of Oatmeal for a Year. Active Scurvy. |
| | 1.03 | | Hospital Diet Plus 30 mg. Cebione Daily for 3 Weeks |
| | 0.69 | | Cebione Discontinued for 10 Days |

Table 6. Patients with subnormal blood values treated with cebione.

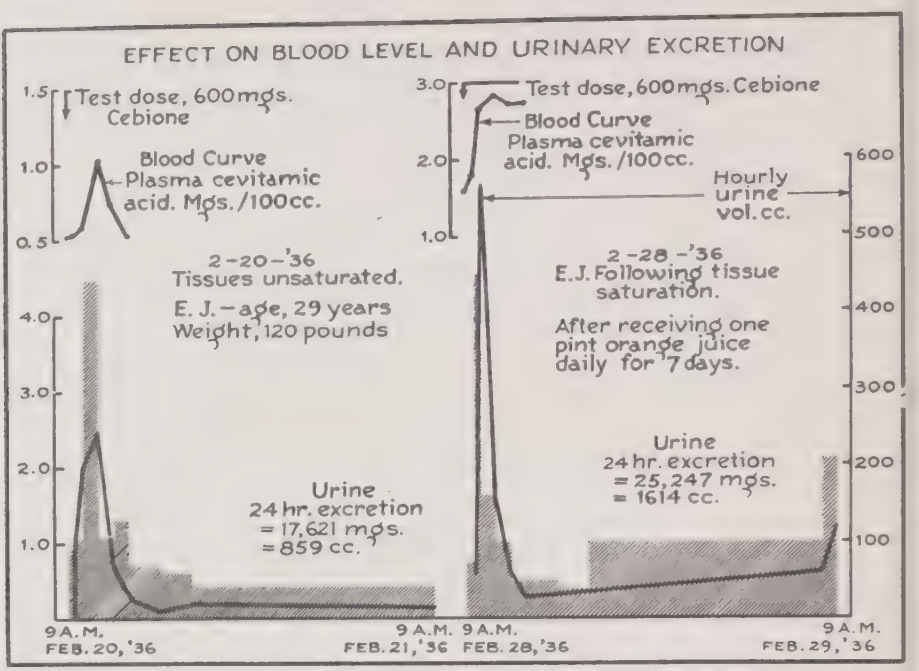


Fig. 4. Tolerance tests—E. J.

dose of 600 mg. of ascorbic acid, the blood was examined at regular intervals for several hours, and the urine for the 24 hours following the test. For the following week the individuals received one pint of orange juice daily. At the end of this time, another dose of 600 mg. ascorbic acid was given and the tests on both blood and urine were repeated. In one instance (Fig. 4), the initial excretion for 24 hours in response to the test dose was 17.6 mg. and after a week of orange juice supplement it did not increase very much, being only 25 mg. In another individual, the initial 24-hour output in the urine, in response to the test dose, was 27 mg. of ascorbic acid; after a week of orange juice supplement it became 32 mg. However, following a second week of orange juice administration, the urinary output for 24 hours became 290 mg. (Fig. 5a; 5b; 5c.). Thus even with the ingestion of adequate amounts of vitamin C it takes a much longer period of time than two or three days to obtain any appreciable excretion of ascorbic acid into the urine. We are of the opinion, therefore, that tissue saturation when judged on the basis of urinary excretion alone, can only be ascertained by following a series of 24-

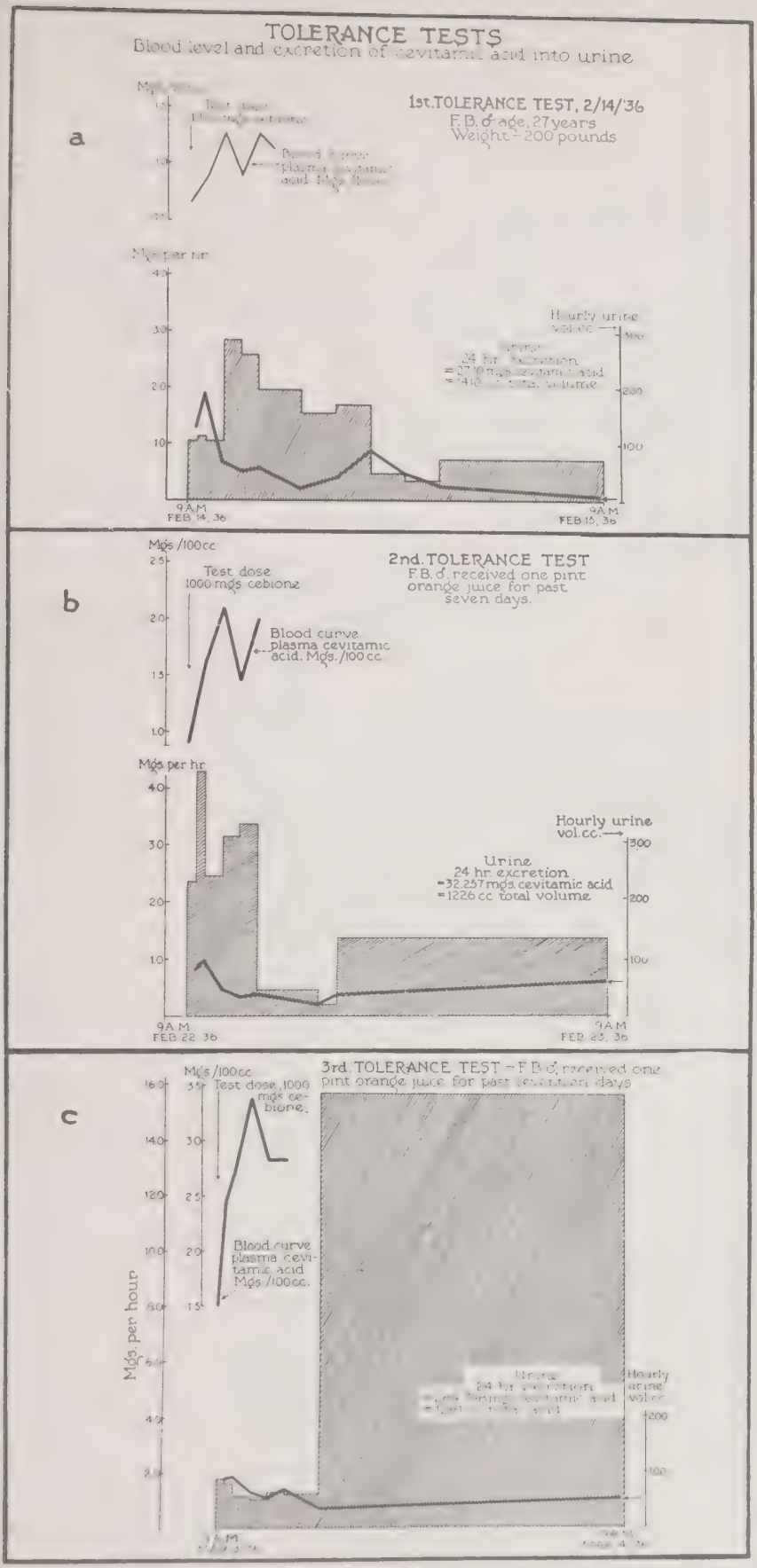


Fig. 5. Tolerance tests. F. B.

hour specimens of urine, frequently for considerable periods of time.

Blood studies conducted on the above individuals as well as many more of a similar nature, have suggested a second procedure for determining tissue saturation which is much more satisfactory than the urine test. It is based upon the use of an appropriate test dose and blood determinations at a 2-hour interval. Following the administration of a test dose of ascorbic acid the initial fasting value of the blood rises rather rapidly over a period of two hours, at which time there may be a drop suggesting absorption or assimilation of ascorbic acid by the tissue, or possible secretion into the urine. A plateau is sometimes observed. Two parts of the curve (Fig. 4 and 5) are of particular interest. If during the week an individual is receiving generous amounts of vitamin C, and his fasting blood level is studied from day to day, it is seen that these values gradually rise. Thus in one individual the initial fasting plasma value was 0.54 mg. per cent ascorbic acid, and after a week on the orange juice supplement, the initial fasting value became 1.2 mg. per cent. Besides the initial fasting levels, it is important to note the height which the curve attains, i.e., the span between the initial value and the 2-hour peak. The test dose of ascorbic acid leads to a rise in the blood usually reaching the peak in two hours. The difference between the initial level and the height attained at the end of 2 hours (assuming normal absorption, and barring abnormal urinary excretion) indicates the rapidity with which the tissues are accepting the ascorbic acid offered to them by the blood. The more saturated the individual is, or becomes, the greater the span between the initial value and the 2-hour peak. On the other hand, individuals who have been on a poor vitamin C diet show a low initial blood value and a short span. After vitamin C therapy for a week or longer, the initial blood level is higher and the span for the 2-hour period, following the test dose, is higher.

It has recently been stated that considerable loss of ascorbic acid is experienced unless potassium cyanide is added with the potassium oxalate when collecting the blood sample. It has been our practice to titrate our samples with the greatest dispatch;

frequently the patient comes to the laboratory so as to obviate any delay. Although immediate deproteinization with subsequent storage of the filtrate at a low temperature allows a reasonable latitude in running determinations, the possibility of keeping blood samples without loss for subsequent deproteinization and titration would be an advantage that cannot be denied. Following the recommendation of Pijoan and associates we added 10 mg. of potassium cyanide along with the oxalate without success. Our results indicate that the cyanide operates in two ways, opposite in direction: by its bleaching action on the dye, it produces an augmentation of the ascorbic acid level of the plasma; by its destructive action on ascorbic acid, particularly upon long standing, it produces an actual lowering in the ascorbic acid level. Under certain circumstances, dependent largely upon the length of time which the plasma stands, these may become compensating errors. At other times, either error may be predominant (Table 7). We have, therefore, adhered to our technic as described above, employing metaphosphoric acid alone.

In an attempt to simplify methods for estimating tissue saturation, Rotter introduced the intradermal test whereby a small amount of the dye is injected beneath the skin and the fading time recorded. We have checked this method against the blood level. Unfortunately no correlation was found between the blood level of ascorbic acid and the bleaching time of the dye.

In our experience, the determination of the capillary fragility under negative pressure shows no relationship to the values for ascorbic acid in the blood, except in scurvy of long standing.

In conclusion, we believe our studies demonstrate the relationship of plasma ascorbic acid content to the dietary intake of vitamin C. The micro-method requires but small amounts of blood, which may be obtained from the finger or lobe of the ear, making venipuncture unnecessary. The method for clinical purposes possesses the advantages of simplicity, accuracy, and rapidity, and may serve as a valuable adjunct in the detection of latent avitaminosis C. It is particularly well adapted to studies requiring the frequent collection of blood samples as in tolerance

tests, or in following the effects of various therapeutic measures. A single fasting plasma determination indicates to a considerable extent the previous dietary history of the individual with respect to vitamin C. If a test dose of ascorbic acid is then administered, and the second determination made after two hours, the span gives further information as to the extent of tissue saturation.

Table 7. The influence of KCN on plasma ascorbic acid values determined by the micromethod of Farmer and Abt.* §

| Time | Plasma Separated and Deproteinized Immediately | Plasma Separated Immediately. Deproteinized at Time Interval Shown† | | Plasma Separated from Whole Blood at Time Interval Shown,†‡ then Immediately Deproteinized | |
|---|---|---|---|---|---|
| | No KCN Mg. Per Cent | No KCN Mg. Per Cent | With KCN Mg. Per Cent | No KCN Mg. Per Cent | With KCN Mg. Per Cent |
| *Blood A* | | | | | |
| Immediate | 1.20 | 1.20 | 1.40 | 1.20 | 1.40 |
| ½ hr. | 1.20 | 1.12 | 1.36 | 1.16 | 1.32 |
| 1 hr. | 1.16 | 1.00 | 1.16 | 1.08 | 1.16 |
| 2 hrs. | 1.08 | 1.00 | 1.08 | 1.04 | 0.96 |
| 6 hrs | 0.76 | 0.60 | 0.60 | 0.68 | 0.56 |
| *Blood B* | | | | | |
| Immediate | 0.64 | 0.64 | 0.80 | 0.64 | 0.80 |
| ¼ hr. | 0.64 | 0.64 | 0.80 | 0.60 | 0.76 |
| ½ hr. | 0.64 | 0.52 | 0.72 | 0.56 | 0.68 |
| 1 hr. | 0.64 | 0.44 | 0.68 | 0.60 | 0.60 |
| 2½ hrs. | 0.60 | 0.44 | 0.64 | 0.52 | 0.60 |
| *Blood C* | | | | | |
| Immediate | 0.36 | 0.36 | 0.64 | 0.36 | 0.64 |
| ½ hr. | 0.32 | 0.28 | 0.56 | 0.32 | 0.48 |
| 1 hr. | 0.28 | 0.20 | 0.52 | 0.28 | 0.40 |
| 2 hrs. | 0.28 | 0.16 | 0.44 | 0.20 | 0.40 |
| 3 hrs. | 0.24 | 0.16 | 0.44 | 0.20 | 0.36 |
| 5 hrs. | 0.24 | 0.12 | 0.36 | 0.16 | 0.32 |

*These data are typical of similar studies conducted upon seventeen different bloods.
†Whole blood to which KCN is added becomes hemolysed after a short period of standing.
‡Bloods differ one from another considerably in the loss of reductive power upon standing.

§ Tables 1, 2, 3, and 7 are reproduced through the courtesy of the *Proceedings of Experimental Biology and Medicine;* and tables 4, 5, and 6 the *Journal of Pediatrics.* Figure 1 is reproduced through the courtesy of E. H. Sargent and Company and the *Proceedings of the Society of Experimental Biology and Medicine.*

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## DISCUSSION

DR. FREDERIC W. SCHLUTZ: We have, of course, not gone into the subject at all as have Dr. Farmer and Dr. Abt and other investigators in the field. We have been primarily interested to know really something about the prevalence of subclinical scurvy. Is it really as prevalent as we were led to think and believe?

We were particularly stimulated not alone by the flood of very excellent investigative work that has appeared since 1931 since the work of Szent-Györgyi, but particularly also by a study of Dr. Park at Johns Hopkins, and also Dr. Wolbach of the Pathology Department at Harvard, on evidences that subclinical scurvy is a reality, is something that we have a good deal more of than we have ever thought or suspected. The work of Park and Wolbach is really very impressive. It was their interesting observations and the availability of the apparently accurate and good methods of Farmer and Abt for cevitamic acid in the blood and the method of Tillmans and King for urine which prompted us to check carefully both our clinical and pathological material during the past year.

We were quite surprised to find in our routine clinical material of the Chicago clinic that we really did not encounter a very high prevalence of subclinical scurvy as measured by these methods. That may not be so surprising if you know something about the economic level from which the material of our clinic is recruited. I am quite sure the situation will be different in other studies that are now under way; for example, in a Negro Hospital in which we have some control of the material. I should also be interested to know what the experience of other clinicians has been in large metropolitan areas—for example, here on your New York East Side—and what may be the experience of Dr. Abt in the Near West Side in Chicago, where we have conditions similar to what you have here, and where there must be, or one would expect to be, a considerable prevalence of subclinical scurvy.

The pathological material of the university clinics in the pediatric department, covering a period of 8 years, has yielded a surprisingly small incidence of this preclinical scurvy—I mean the material analyzed in the light of the very careful criteria laid down by Dr. Park and Dr. Wolbach. We are extending this investigation to a larger hospital in which the university's pathology department has for a number of years conducted pathological work, and which serves a

group which is at an entirely different economic level from the standpoint of nutrition and living conditions. What this may show I am not yet prepared to say, but presumably we will find a higher prevalence. We are, after all, interested in the practical application of a lot of this excellent work, and a demonstration of a really increased prevalence of this condition which in turn, of course, will lead to the application of measures of prevention.

The blood method that Dr. Farmer has presented seems to be one of the best ones just now available—that, and the method for urine by Tillmans modified by Bessey and King apparently will give accurate data which will enable one to determine the levels at which the subclinical scurvy is apt to appear.

Just what this threshold is and where subclinical scurvy or asymptomatic scurvy becomes symptomatic, I do not think is definitely known. There seems to be variation in the figures reported, and such experience as we have had would lead us to say that just now no one knows where the level is. It is extremely important, of course, to discover that particular critical point.

I recall one instance in the very excellent study made by Ingalls at Harvard where he required for his infants intakes of 2,000 milligrams of vitamin C to approach to any degree what you do not like to call the saturation level.

That, of course, is an enormous amount of orange juice, I think, close to .4 liters. Who ever gives such an amount? We have always been able to cure scurvy with an infinitesimal part of that, and very readily, so you see there is much conflicting data and a good deal of confusion in the field.

Dr. Elaine P. Ralli: I should like to digress from the childhood evidences of scurvy to the adult, because I know very little about children and do not work with them.

First, let me say that we have found the same results with potassium cyanide as did Dr. Farmer. I think it is unnecessary to repeat the data. Secondly, I should like to speak briefly, but not as an authority, on the kidney. We have done simultaneous vitamin C and inulin clearances. An analysis of the data shows that the vitamin C clearance has a low value when the plasma vitamin C is below 2 milligrams per cent. As the plasma level is raised the vitamin C clearance rises rapidly and approaches the inulin clearance as a limiting value. From the experiments it was found that vitamin C was excreted by

glomerular filtration and active tubular reabsorption and the reabsorptive mechanism is limited by a maximal rate (an average of 2.5 milligrams per minute). When the vitamin is presented to the tubular by the glomerular filtrate at a rate exceeding this maximum, the excess is excreted in the urine. It follows that the quantity of vitamin C excreted in the urine of an individual will be determined by the plasma level, by the rate of glomerular filtration and by the rate of tubular reabsorption. There is therefore no absolute threshold for vitamin C.

The other points I want to mention are from the public health point of view. We are certainly very grateful to Dr. Farmer for giving us a satisfactory blood method. It has made it possible to consider the question of vitamin C deficiency objectively. The normal range is quite well defined. The question comes immediately as to what is scurvy in our present knowledge, and what is subclinical scurvy? I do not believe that one can say that at any definite plasma level the symptoms will appear.

We have been studying the nutritional status of individuals over 60 years of age in one of the chronic clinics. These patients all happen to have arteriosclerosis. We found that of 22 cases studied up to the present, 20 have vitamin C blood levels so low that theoretically they should have scurvy. We will either have to define what we mean by clinical scurvy, or we will have to admit that at a certain blood level we have a condition which should be called either preclinical scurvy or latent scurvy, or simply a low vitamin C state of nutrition. That, I think, is an important point of view to consider, because by the time you get the symptoms of scurvy you have gotten the pathological changes, and they have not changed much since they were originally described.

This other group, it seems to me from the public health point of view, deserve to be considered as not being normal, certainly not when it comes to vitamin C. The normal, well-nourished individual on an adequate intake will have a blood level in the neighborhood of 1 milligram per cent. The patients at Bellevue Hospital in New York City are usually not very well nourished, so we are dealing with the poorly nourished, and in taking a series of bloods on the wards we can count on finding 80 per cent at the lower level, regardless of age. In the adult ward we have patients from 16 years on, and we definitely find that the majority of patients will run a low plasma vitamin C.

As far as giving toxic doses of vitamin C are concerned, we have given 6 grams intravenously, and have had absolutely no toxic effects.

I wonder if there is any toxic effect from the water-soluble vitamins. Nobody has observed it in vitamin $B_1$, and I think 6 grams is a critical dose of vitamin C. This we gave in order to maintain a blood concentration, and we reached a concentration of vitamin C in the blood of 20 milligrams per cent, which can only be reached by giving large doses intravenously. The rise in the blood of vitamin C occurs within a period of 15 to 20 minutes, which is the peak, I think, and which is approximately the mixing time; and all of the test doses that have been used for urinary excretion obviously depend somewhat on the level to which the vitamin C is raised in the blood. We found that in an individual the blood vitamin C was raised from about 1.5 milligrams per cent to over 2 milligrams per cent by the injection of 100 milligrams of cevitamic acid and during that period there was a very definite excretion in the urine.

I still think that these excretory test doses are a good measure of the state of vitamin C nutrition. The level of the blood vitamin C can also be taken as an index of the existence of the scorbutic state in an adult individual, and by following the blood one is in a position to state whether the person has reached a normal state.

I think the word "saturation" has to be carefully defined. I am not at all sure whether one should continue to use it. There may be a lot of implications in that word, and I should think that we should be better off, perhaps, in speaking about the blood levels which we now can establish, and perhaps readjusting our point of view on the nutritive state of the individual, as far as vitamin C is concerned, on the basis of the blood level and the response to vitamin C which is administered by mouth or otherwise.

We have found that the amount of vitamin C necessary to return an individual with the symptoms of scurvy to a normal state depends on certain factors. One of them is the duration of the deficiency diet, and another is the presence or absence of any complicating disease.

Dr. Arthur F. Abt: There is this to be said about blood levels which would show the prescorbutic asymptomatic state. From our results it may be stated that there is probably an individual variation in the amount of vitamin C in the body when the symptoms of scurvy may present themselves. In certain individuals you may get a blood of 0.5 mg. per cent and have evidence of active scurvy. In other in-

dividuals, the blood may go to 0.2 mg. per cent before there is evidence of scurvy. None of our scurvies has been at exactly one blood level, so why should there be any special blood level for prescurvy, if there is such a state? I think there is an individual level for the development of symptoms of scurvy; therefore, may there not be an individual level for presymptomatic scurvy? But after all, what we are interested in is the optimum level. We want to get away above those low levels and give the mass of our population really a more than sufficient dietary, a dietary more than enough to prevent any possible pathologic level.

Little is known concerning assimilation. To date none of the reports which we have read concerning metabolic studies with vitamin C have considered assimilation, absorption, and excretion through the intestinal tract. Professor Farmer and Dr. Herman Chinn have studied the problem of absorption of ascorbic acid from the gastrointestinal tract. They have devised a method for the estimation of ascorbic acid in the feces, and have been able to demonstrate that the normal fecal content is approximately 5 mg. per day.

Theoretically, decreased absorption in the intestinal tract may result from increased fecal excretion due to increased peristalsis, as in diarrhea, or there may be destruction of ascorbic acid in the intestinal tract before absorption is possible. They have been able to demonstrate in a limited number of patients suffering with actual disease of the gastrointestinal tract that the fecal content for ascorbic acid was much increased over normal. Simultaneous blood and urinary values were lower than might be expected for the amount of oral intake of vitamin C. This clearly demonstrates a failure of absorption and shows the importance of considering the gastrointestinal tract and fecal excretion of the vitamin.

Furthermore, they found that the blood ascorbic acid in a number of achlorhydric patients was significantly lower than in normal individuals on a similar dietary. From the experimental work performed it may be suggested that a combination of factors, such as bacterial action, alkalinity, and malabsorption explain the low blood values in these achlorhydric patients. This careful experimental study stresses the importance for considering malabsorption of vitamin C from the gastrointestinal tract. May there not be apparently normal individuals whose absorption is interfered with? There are also probably numerous other pathologic conditions which interfere with absorption. These phenomena may only be elucidated

when the factor of abnormal absorption is thought of and careful fecal determinations, in addition to blood and urinary determinations, are made.

I may, perhaps, refer to Rotter's intradermal test; we find that there is no very definite correlation between the blood level and the bleaching or fading time of the dye in the skin. The idea was to inject a small amount of the dye, just as one does in a Schick test or any intradermal test, and observe the time that it took the dye to fade. In other words, the skin would give a direct titration of the dye and, of course, this would be widely applicable because of its simplicity.

We checked the intradermal method against the blood level. With my blood of 0.8 mg. per cent, the dye faded in 4 minutes and 40 seconds. With a blood of 0.9 mg. per cent, it took 11 minutes. With a low blood of 0.48 mg. per cent, the dye faded in a shorter time (7 minutes). With another blood, which was practically comparable with mine (0.84 mg. per cent), it took 4 minutes and 45 seconds.

In one young man the fasting blood level was around 0.4 mg. per cent, with a fading time of 4 minutes. We gave him 450 milligrams of ascorbic acid by mouth and did simultaneous blood and skin tests. In the second determination in a half hour, his blood went up to 0.5 mg. per cent, while the skin still faded in 4 minutes. Then his blood went up to 0.8; and the skin did not fade until after 9 minutes; and the higher the blood level, the longer became the fading time. With this apparently very simple test, we could not therefore obtain any consistent correspondence with the blood levels.

Numerous reports imply an augmented vitamin C metabolism in many infections. There is no proof that the vitamin is absorbed and utilized in any of these infections. I should like to raise the point: What is the cause for the failure in oral use, when parenteral use gives positive results? Why do we get positive results with extremely large doses where small doses are unavailing? I cannot explain that, but it seems to be the fact.

In conclusion, I should like to agree with Dr. Schlutz. Our first levels were taken from the ordinary dispensary patients who were in a fairly good economic state, but since we have been working at the Municipal Contagious Hospital—that is, the West Side of Chicago—where most of the patients are very poorly off economically, we have found that their initial blood levels are considerably lower than the values which we obtained from our dispensary group.

Dr. Chester J. Farmer: We have recently developed a method for estimating vitamin C in feces. It is briefly as follows: the freshly excreted material is made into a sludge with metaphosphoric acid. The suspended material is thrown down and most of the interfering substances removed by the addition of lead acetate. After centrifugation, an aliquot of the clear supernatent fluid may be titrated for total reducing substances with 2,6 dichlorophenol-indophenol. A second aliquot is incubated with a vitamin C oxidase obtained from squash or cauliflower, which destroys the vitamin C present. After destruction of the vitamin, the indophenol titration represents nonascorbic acid reducing substances present. The value for these substances is then subtracted from the titration of the original aliquot, the remainder representing the ascorbic acid present. The amount present in feces is ordinarily very small; normally 1 to 5 milligrams in the total 24-hour amount. However, in one or two cases we have found amounts as high as 20 to 60 milligrams, but these were attended by diarrheal conditions.

# HEMATOLOGIC METHODS IN DETECTING NUTRITIONAL ANEMIA

G. M. GUEST[1]

IN CONSIDERING nutritional anemia, it would seem advantageous to present evidence first on the variability in characteristics of red blood cells (their number, size, and hemoglobin content) and in amounts of hemoglobin in the blood that may be found among large groups of infants at different ages; and then to point out its bearing upon certain specific problems of the anemic state. The data to be presented first have been derived from over 1,000 blood samples, drawn from more than 600 white infants at ages from birth to 5½ years, who before the blood samples were taken had never received iron other than that in their diets. The observations are assembled from a wide range of different groups and types of individuals, healthy and otherwise, anemic and nonanemic, but excluding prematurely born infants and those with primary blood dyscrasias. The data will be presented graphically in scatter charts and frequency distribution histograms for the purpose of showing the age periods in which notable changes in character of the erythrocytes were found, the directional trends of the changes which occurred at different ages, and the relative frequency with which varying degrees of anemia existed among infants in various age groups.

The record sheets which we devised for tabulating these data on individual infants suggest not only the general program of the studies but also the plan for statistical analysis of some of the factors which may affect the variability in these blood data. The individual record sheet is planned for transference of the data to punch cards. Provision is made for weights and correlating such factors as the following, named in the order in which they are listed on the cards: (1) General information—color and sex;

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group (institutional or family); birth date (year and month, for seasonal variation); birth weight; term of gestation (full term or premature); term of breast feeding; mother's age; birth order of this child; interval since previous child. (2) Information pertaining to the time of the blood sample—year and month; age in days; weight; height; the blood data; and health of the child. Attempts were made to list other data pertaining to the state of the child, such as teeth, X-ray of bones, history of pica, etc., but it is doubtful that these data will find use.

### VARIABILITY IN BLOOD VALUES OF INFANTS

Figure 1, displaying values on the blood of a normal 4-year-old child, presents the terminology we have employed. Blood samples were all obtained by venipuncture, various superficial veins being used. The blood was transferred from the syringe to a small vial containing dried heparin in suitable amount, and the determinations listed here were carried out on this heparinized blood: volume of packed cells; cell count; and hemoglobin

Fig. 1. Data on blood of a normal 4-year old child, selected as an example to illustrate the terminology and derivation of the cellular values.

| METHOD AND TERMINOLOGY<br>Blood of a normal 4 year old child | |
|---|---|
| **VALUES DETERMINED for WHOLE BLOOD:** | |
| A. R.B.C. COUNT - millions per c.mm. | 4.69 M |
| B. VOLUME OF PACKED RED CELLS - per cent | 39.3 % |
| C. HEMOGLOBIN - grams per 100 cc. | 13.5gm% |
| **VALUES CALCULATED for RED CELLS:** | |
| MEAN RED CELL SIZE $\frac{B \times 10}{A}$ - cubic microns | 84.0 $\mu^3$ |
| Hb CONCENTRATION $\frac{C \times 100}{B}$ - grams per 100 cc. | 34.4gm% |
| MEAN Hb CONTENT PER CELL $\frac{C \times 10}{A}$ - micromicrograms | 28.8 $\gamma\gamma$ |

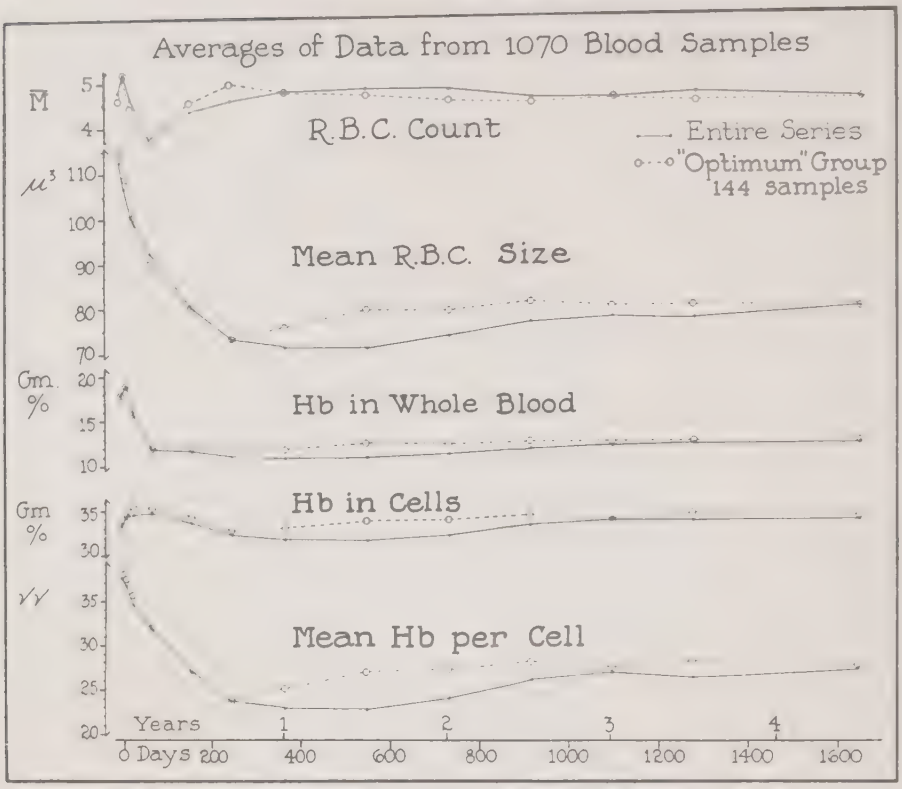



Fig. 2. Averages of data on the 1,070 blood samples represented in the scatter charts 3, 4, 5, 6, and 7. The broken lines represent averages of data on 144 blood samples from an "optimum" group of infants reared under especially favorable circumstances in families of upper economic levels.

content of the whole blood. From the three values thus determined, three additional figures describing the characteristics of the cells were calculated: mean red cell size; hemoglobin concentration, and mean hemoglobin content per cell. It may be noted that this terminology differs somewhat from that commonly used at present by many hematologists. For example, the terms introduced by Wintrobe designate the latter three values as: mean corpuscular volume; mean corpuscular hemoglobin concentration; and mean corpuscular hemoglobin. One should, perhaps, apologize for introducing terms different from those which are in current usage. However, in my own experience the use of the "corpuscular" terms frequently leads to much confusion; hence I finally chose the shortest words which seemed to fit the needs of the descriptions.

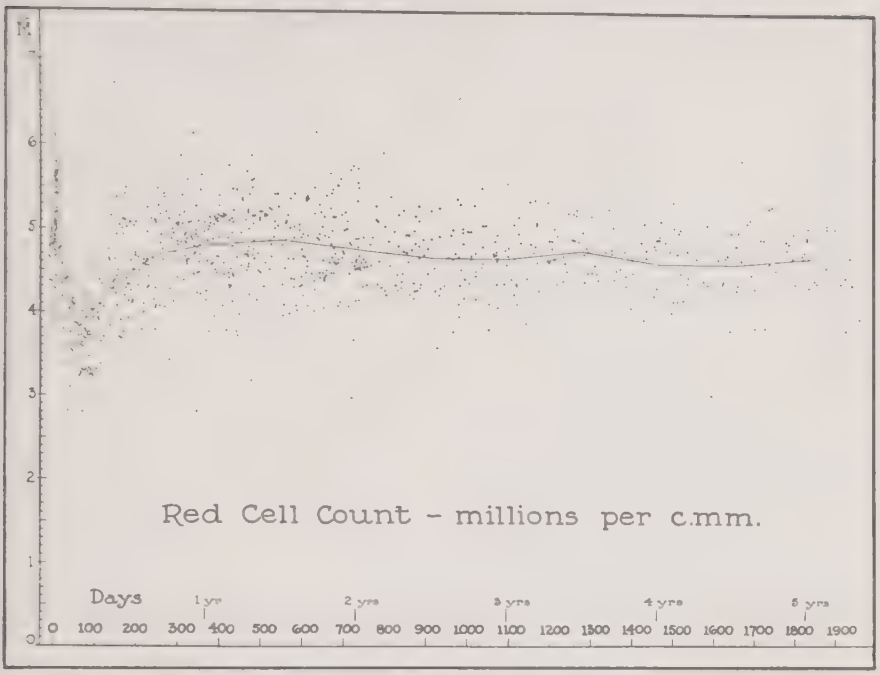



Fig. 3. Red blood cell counts. In this and the following scatter charts, the solid line represents the averages of values found in the different age periods; these averages being represented also in Figure 2.

Figure 2 shows the averages of data on 1,070 blood samples drawn from infants during the age period from birth to 5½ years. Averages of data on 144 samples from an optimum group of infants are indicated by the dotted lines. This group of well infants are referred to as optimum merely because they came from families of the higher economic levels, were reared under favorable circumstances, and have been relatively free from nutritional disturbances and acute infectious diseases. These infants came from the private practices of some of our medical colleagues, and many of them from their own families. Figures 3 to 7 present scatter diagrams showing the distribution of the individual values from which the average curves of Figure 2 were prepared.

*Red Blood Cell Count.* The mean of the cell counts in cord blood samples was 4.8 millions. Although this figure is not as high as has been reported in older literature, it is in agreement with contemporary reports on the cell counts of newly born infants.

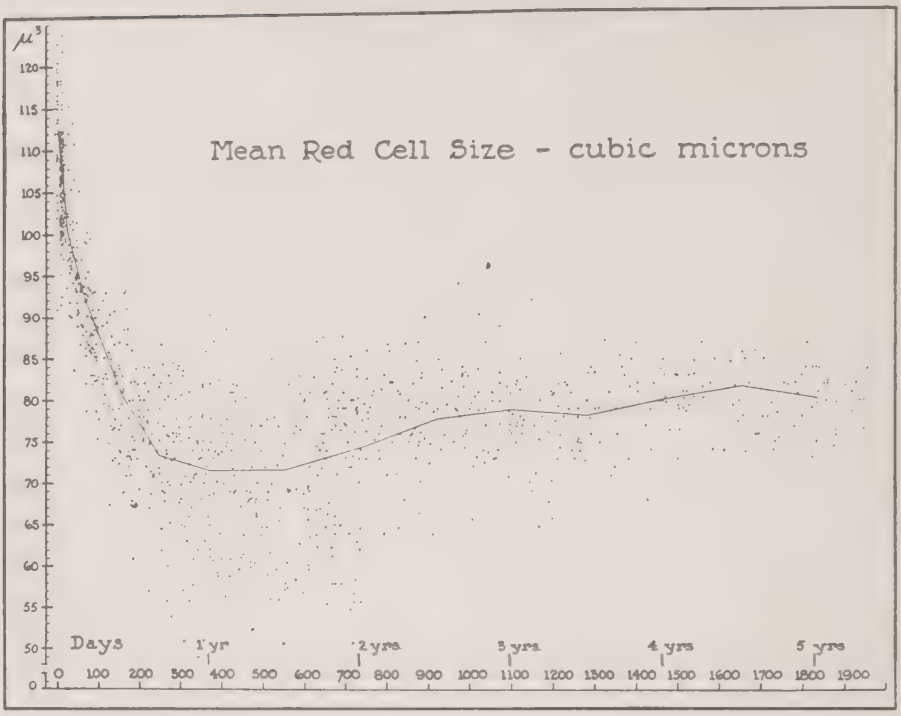



Fig. 4. Mean red blood cell size.

During the first few days of life the number of cells tended to increase. Such increases may have been due in part to dehydration in some of the infants who received too little fluid during this period. After the first 10 days or so, the cell counts diminished rapidly for 2 or 3 months. Such a fall in cell count is most exaggerated in prematurely born infants and is the most significant factor in the blood picture of the early anemia of prematurity. It should be stated, however, that data from prematures are not included in these charts. After about 2 months the cell counts increased, and attained a mean value of 4.8 millions at the end of the first year, remaining approximately at this level during the remainder of the 5-year period and through childhood. The greatest variability (widest scatter) is found at ages from 6 months to 18 months; this is less marked after the end of the second year, and decreases further in the fourth and fifth years.

*Red Blood Cell Size.* The red cells at birth were very large, but decreased rapidly in size until at about 6 months the average was

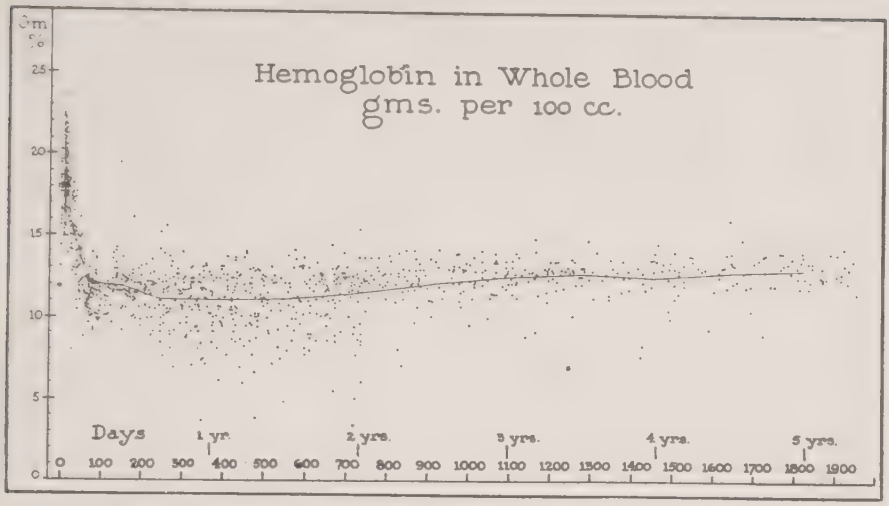



**Fig. 5. Hemoglobin in whole blood.**

around 75 cubic microns. At about 8 months, values below 70 cubic microns began to appear, and thereafter increased in frequency through the next 6 months, or most of the second year. The infants with values below 60 cubic microns often enough had clinically recognizable anemia, but it is important to note here that anemia had not been suspected in a great many of the infants whose red blood cells were found to be this size or smaller. Since the frequent development of such a degree of microcytosis was limited to the ages between 8 and 27 months, this may be properly described as the most critical age period for the development of microcytic anemia.

*Hemoglobin in Whole Blood.* The hemoglobin in whole blood, expressed as grams per 100 cc., was high at birth. After the first few days the average values decreased rapidly for about 2 months, this change corresponding to the decrease in cell count already noted for this period. Thereafter the average values decreased more slowly until the eighth month, after which time they remained fairly level until after the middle of the second year. It should be noted that the low values, indicating severest anemia, were found most frequently in the period from 8 months to 2½ years.

*Hemoglobin Concentration in the Cells.* There was a fairly marked variability in the values for hemoglobin concentration

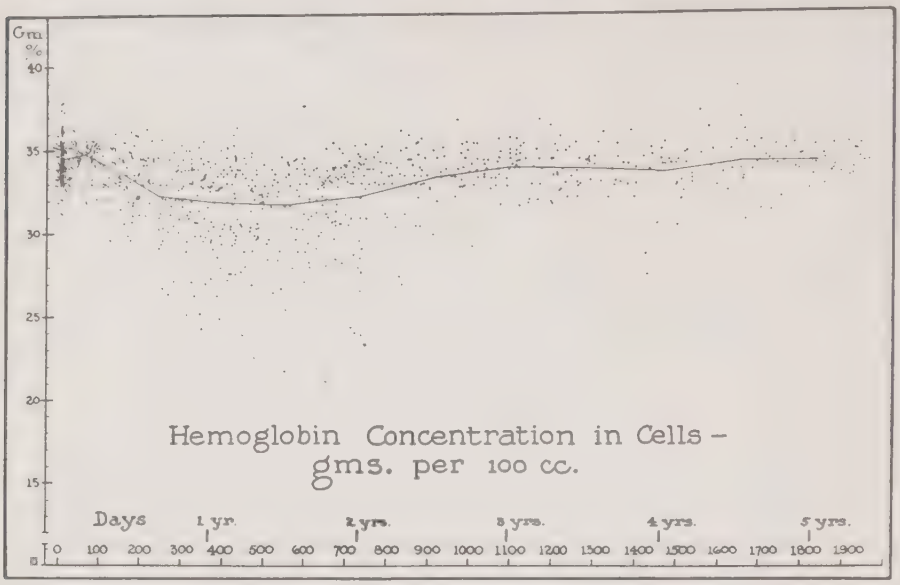



Fig. 6. Hemoglobin concentration in red blood cells.

in the cells at birth, shown on Figure 6 by the wide scatter at that time. This scatter tended to diminish during the first 3 months, and the average values actually increased during that time. Thereafter the average figures decreased, and the lowest averages were found during the second year. Values below 28 grams per 100 cc., such as may be found commonly in moderately severe anemia, were practically limited to the age period between 8 months and 2½ years, which may therefore be described as the most critical period for the development of hypochromic anemia.

*Mean Hemoglobin Content per Cell.* The values for the mean hemoglobin content per cell are, of course, affected by changes in both size and hemoglobin concentration of the cells. During the first 6 months of life, the mean size of the cells decreased, in most instances without a significant decrease in the hemoglobin concentration of the cells; but after 6 months the tendency to both microcytosis and hypochromia contributed to the increasingly frequent occurrence of low values for the mean hemoglobin content per cell which was most pronounced in the middle of the second year. Since changes in size and hemoglobin content are both in the same direction during this age period, for practical clinical purposes this value alone serves most needs for diagnosis

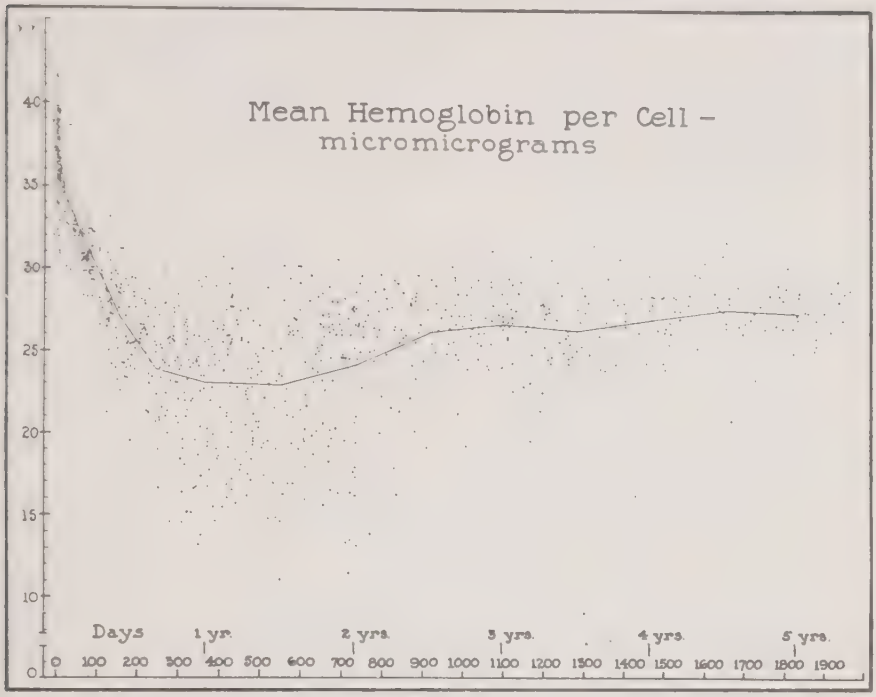



**Fig. 7. Mean hemoglobin content per red blood cell.**

and control of treatment. The distribution of low values in Figure 7 again delineates the stretch from 8 months to 2½ years as the most critical age period for the development of microcytic hypochromic anemia in the majority of these infants.

Presented in Figures 8 to 12 are histograms showing the frequency distributions of these six kinds of values, according to the different age periods. The trend in values for hemoglobin in whole blood through the different age periods, as given in Figure 10, is of especial significance. At any age, values below 10 grams of hemoglobin per 100 cc. of blood may be assumed to indicate a moderate to severe degree of anemia. At 18 months, the significance of a value around 11 grams, the average for the whole group at this age, in an individual infant may be difficult to gauge. This value certainly does not represent a level that can be accepted as normal, even though it would be accepted as within normal limits in most clinical practice. It is probable that the value 12.5 grams, shown in Figure 2 as the average in the optimum group at 18 months, may be considered desirable.

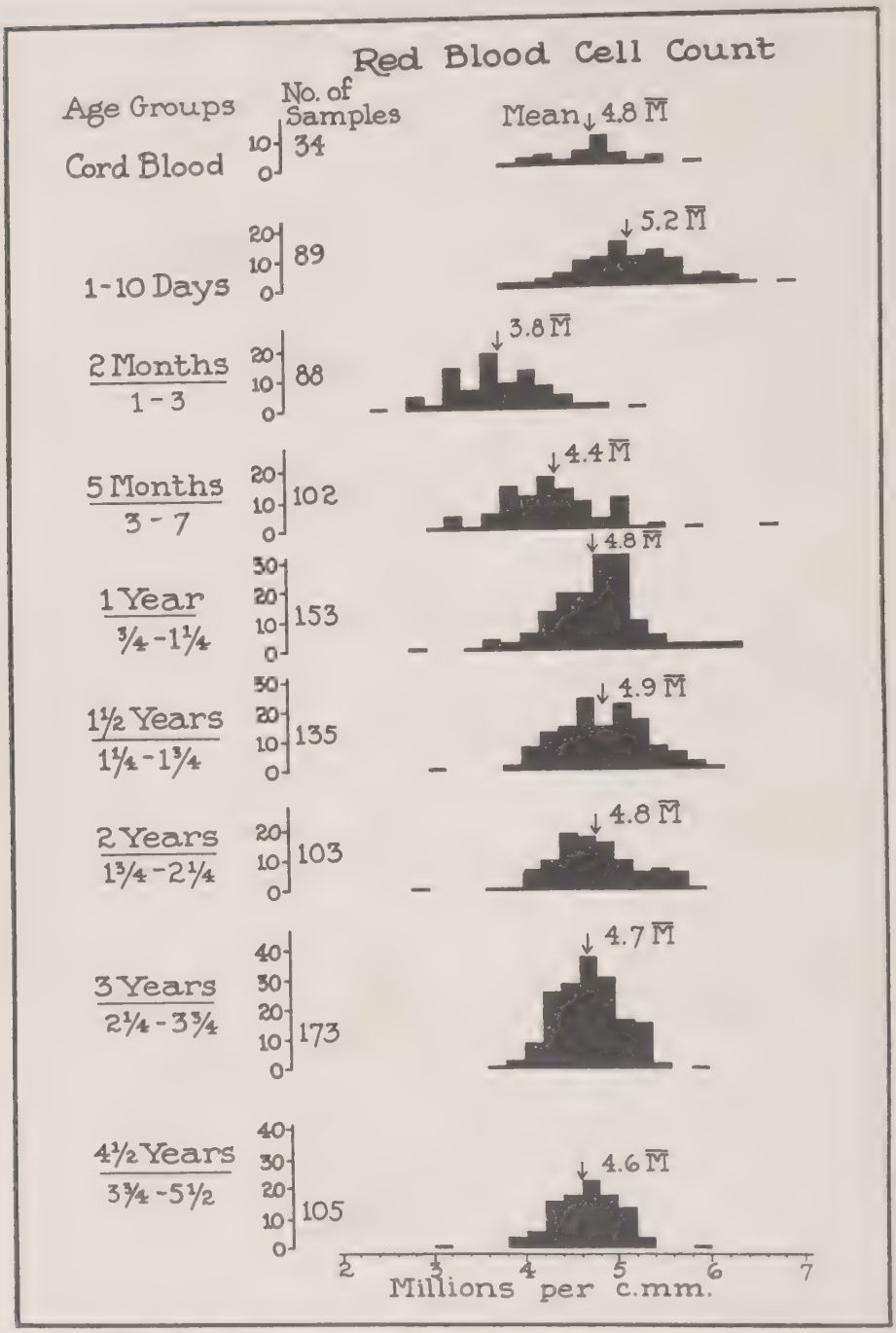



Fig. 8. Red blood cell counts. Frequency distribution of values found in different age periods.

In any case, the distribution of values below such levels may be

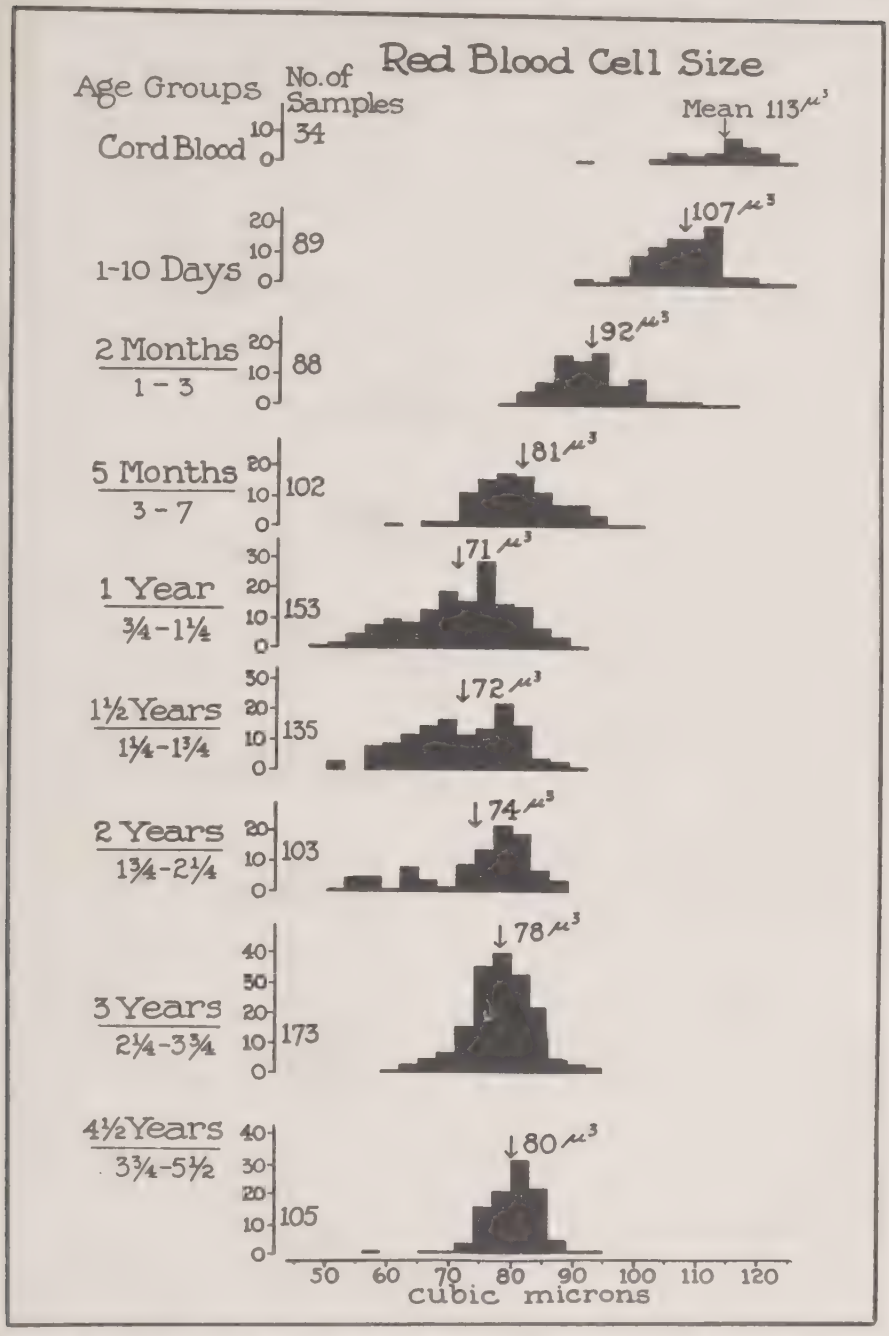



Fig. 9. Mean red blood cell size. Frequency distribution of values found in different age periods.

cited to demonstrate the relative tendencies shown by these in-

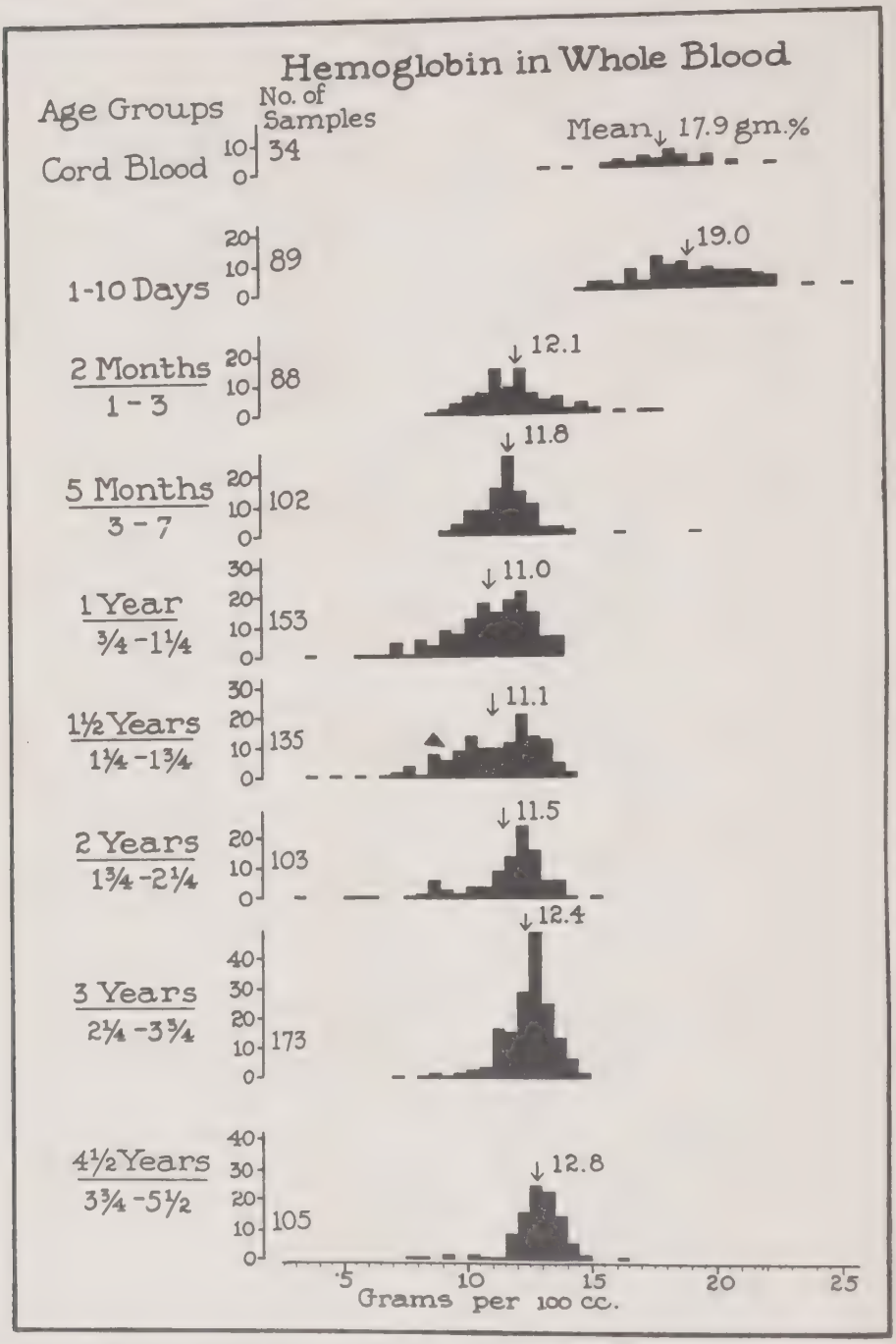



Fig. 10. Hemoglobin in whole blood. Frequency distribution of values found in different age periods.

fants to develop varying degrees of anemia at these ages. The

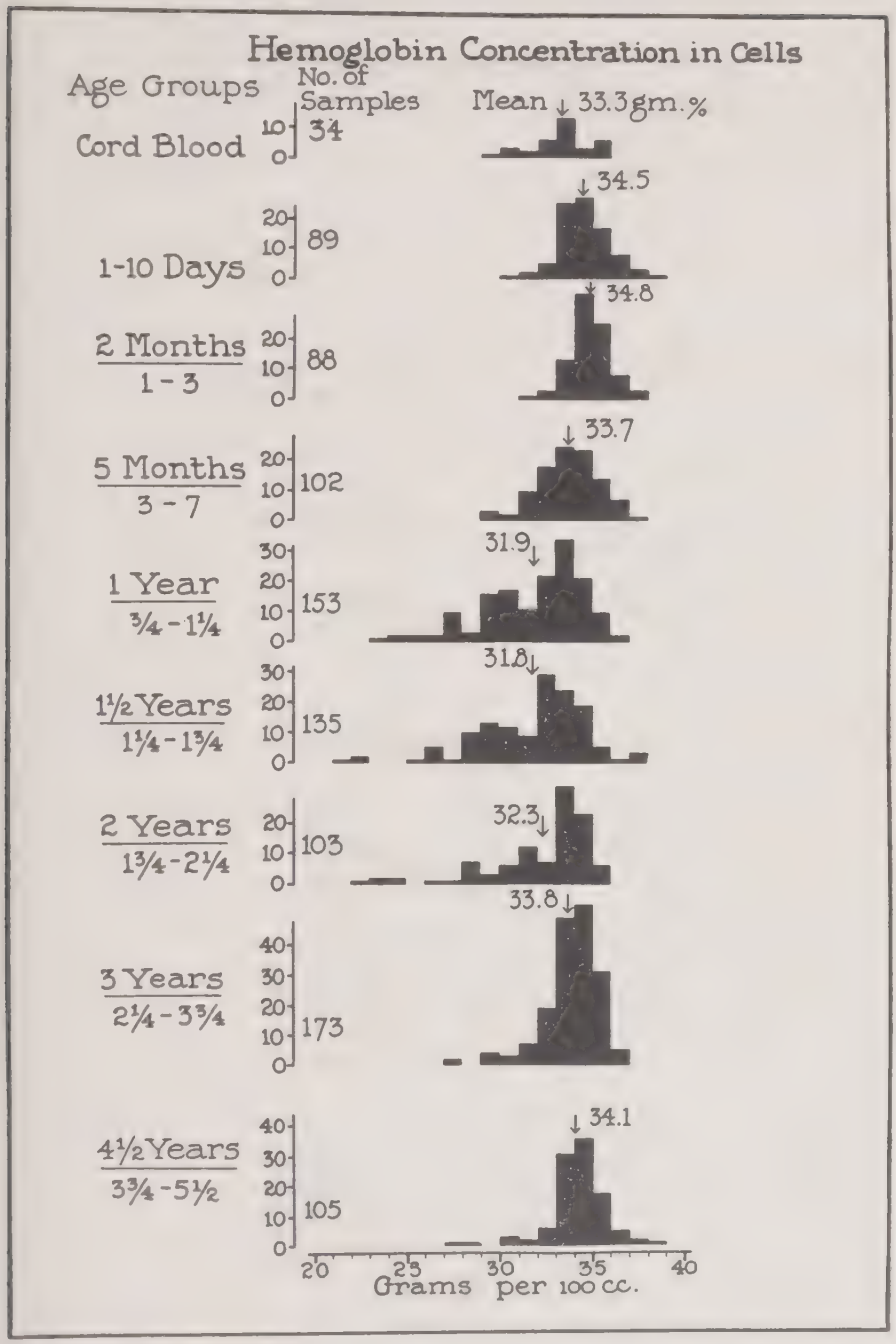



Fig. 11. Hemoglobin concentration in red blood cells. Frequency distribution of values found in different age periods.

changes in number, size, and hemoglobin concentration of the

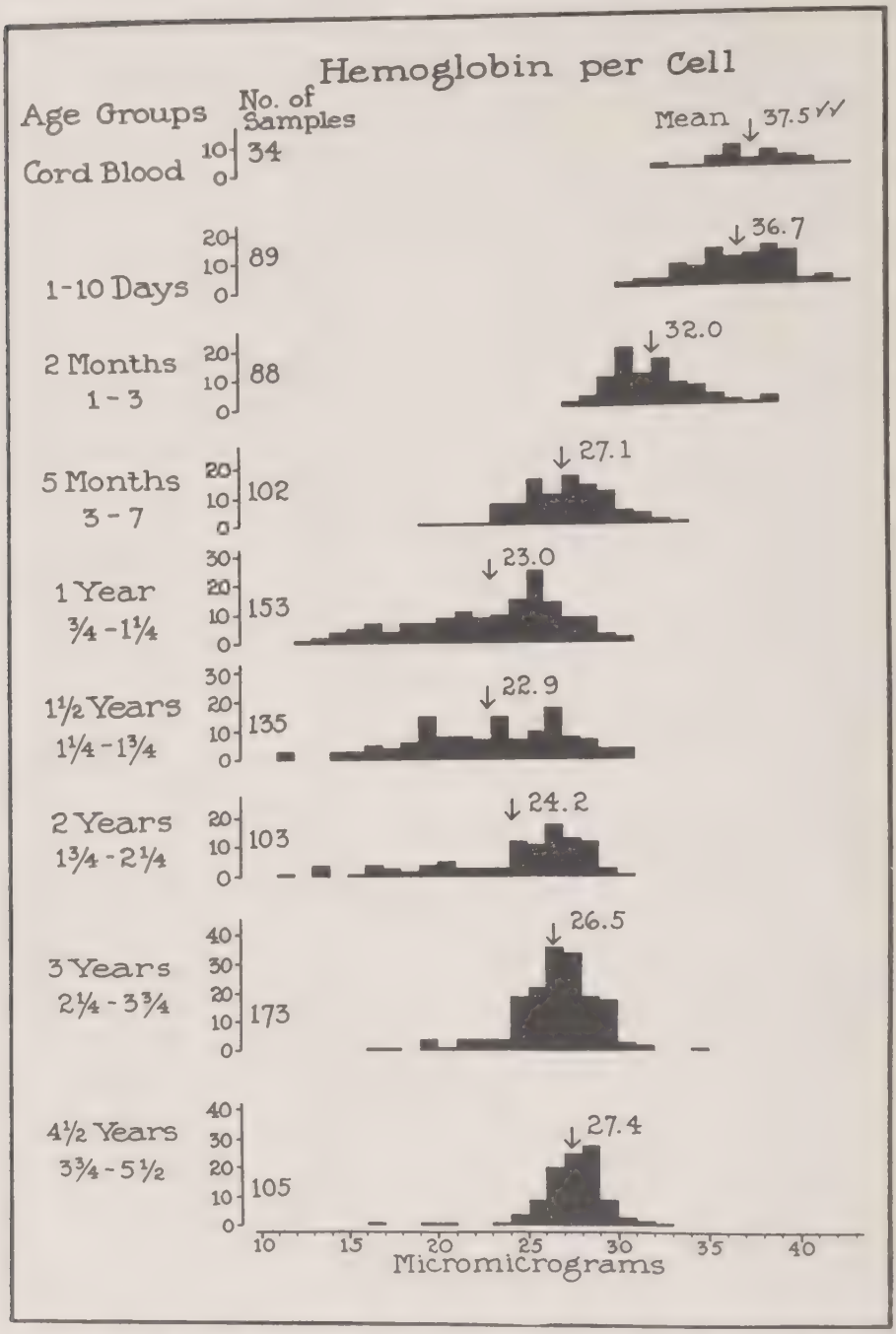



Fig. 12. Mean hemoglobin content per red blood cell. Frequency distribution of values found in different age periods.

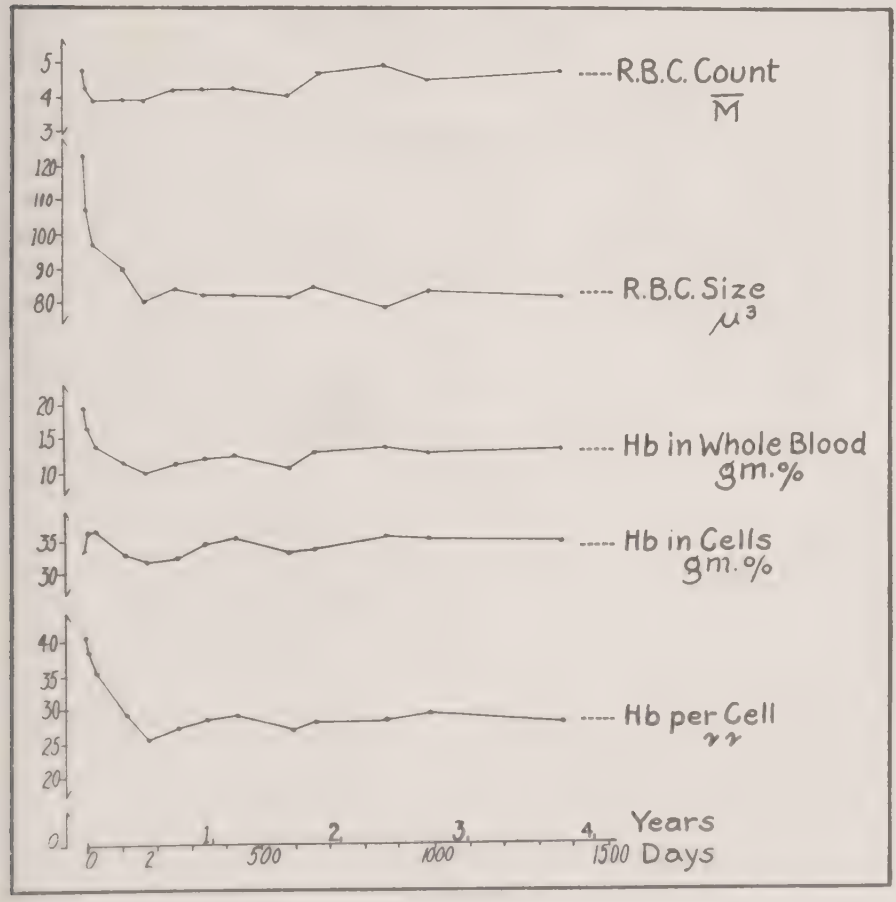
red cells complement each other in affecting the amounts of

hemoglobin contained in the whole blood. These separate factors should therefore be kept in mind while examining these figures. Values below 10 grams occurred most frequently at ages from 8 months to 2½ years, when the cells decreased most in size and hemoglobin content but not necessarily in number. Values below 8 grams were practically all found in the latter part of this age period.

## NUTRITIONAL ANEMIA

The blood in the so-called nutritional anemia, or iron deficiency anemia, is characterized by normal, high, or low red cell counts; a low hemoglobin content of the whole blood, although this need not be marked; pronounced microcytosis and hypo-

Fig. 13. Changes observed in the blood of a white boy, apparently normal in general physical development, from birth to the age of 3½ years.



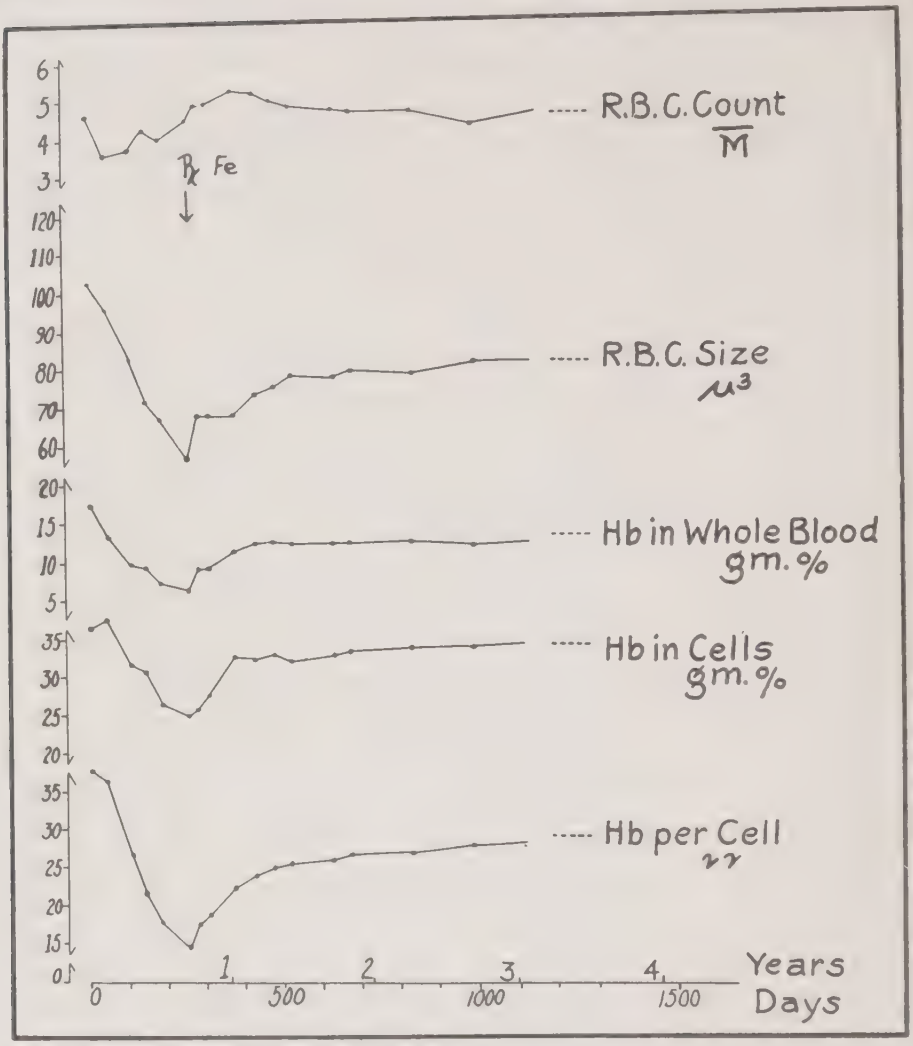



Fig. 14. Changes observed in the blood of a negro boy, apparently normal in general physical development, from birth to the age of 3¼ years. Curves illustrate the development of anemia, and the recovery after the administration of iron.

chromia of the red cells. That appropriate iron therapy leads to improvement in these values in anemic individuals need not be argued here, but the records presented in Figures 13 to 17 furnish some information as to when and why such therapy should be offered in individual cases.

Figure 13 demonstrates changes observed in the blood of a normal white male infant, from birth to 3 years of age. This infant received no iron other than that in his diet. The blood

changes follow a pattern fairly similar to that of the average of the "optimum" group shown in Figure 2. This record is cited to show the fluctuations that may be commonly observed in a "normal" individual, and serves as a basis for comparison with an obviously abnormal pattern of changes shown in Figure 14.

Figure 14 demonstrates the sequence of changes observed in the blood of a negro male infant, during the development of and recovery from a fairly marked degree of anemia. This infant was examined frequently in the outpatient clinic, and a varied diet was recommended early. His weight and physical development appeared to be normal at all times. The size of the red blood cells decreased progressively until the eighth month when they reached a mean size (57 cubic microns) as small as that of the cells found in the blood of patients with clinically recognized severe nutritional anemia. The hemoglobin of the whole blood fell to 6.5 grams per 100 cc.; the hemoglobin concentration in the cells, to 24 grams; the mean hemoglobin content per cell, to 14 micromicrograms. At eight months iron therapy was started, and this was followed by a progressive increase in all of these values.

Figure 15 presents data from 8 cases of typical nutritional anemia, ranging in age from 10 months to 2½ years, showing the initial findings in their bloods, and the changes which occurred after treatment with iron was begun. Iron therapy in these cases was followed by an initial increase in cell count, followed by a drop to the average value of 4.5 millions, rapid improvement in values for hemoglobin in whole blood and in values for size and hemoglobin content of the cells. The pattern of the changes following treatment in the several cases may be seen to be quite similar, at whatever age the treatment was begun.

Interesting observations on the familial aspects of nutritional anemia have been made on sets of siblings from two mothers (Fig. 16 and 17). These two families have been followed closely for several years, and certain sections of the data taken from the records of each child are plotted together on the same chart in order to compare the blood changes each developed at different ages. These studies are still in progress, and the last values—for

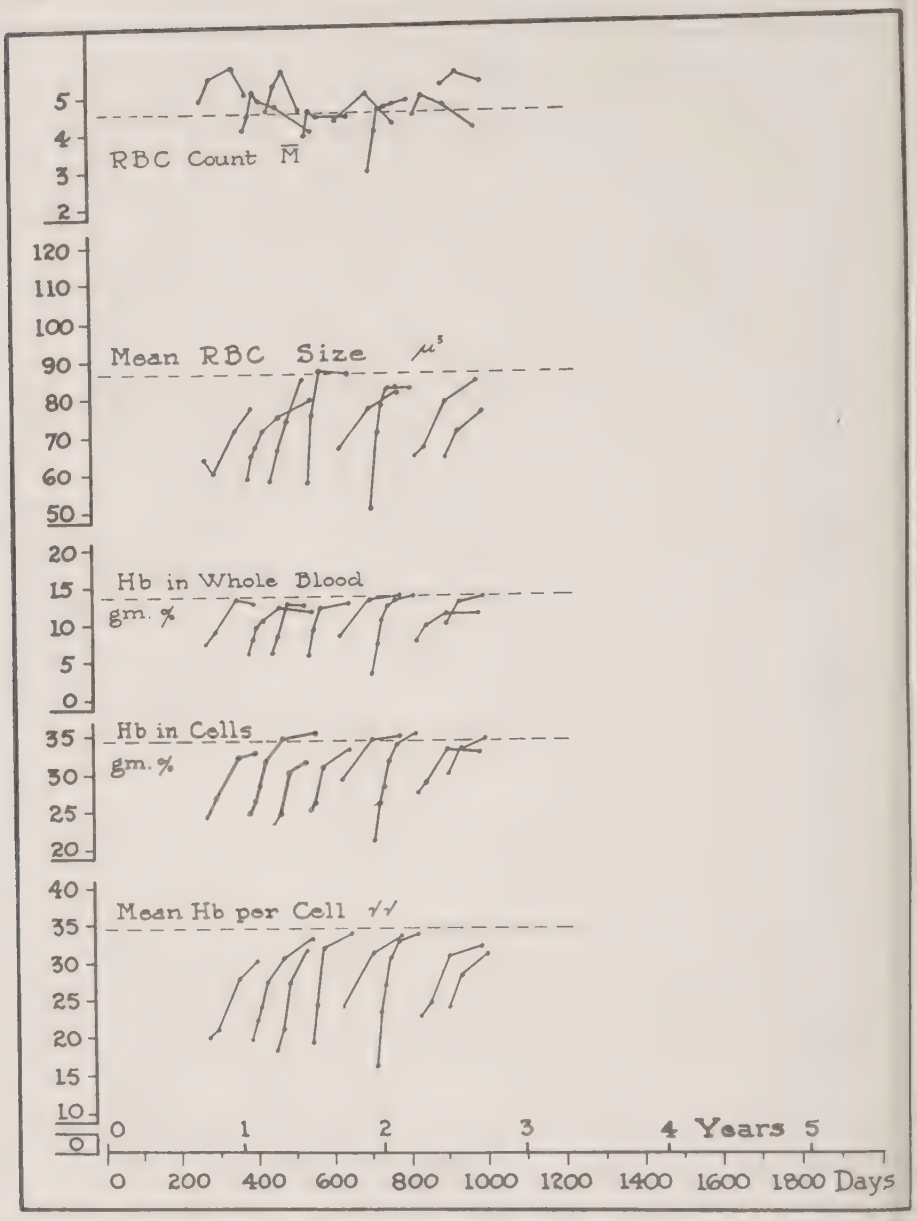



Fig. 15. Effects of iron therapy in nutritional anemia. Changes in the blood of 8 anemic infants of different ages, following the administration of iron.

the youngest infants—were added to the charts early in March. More will be added later for future publication of these records.

Of the first family, siblings no. 3, 4, 5, and 6 are represented in Figure 16. The fourth sibling, a boy, was the first of this

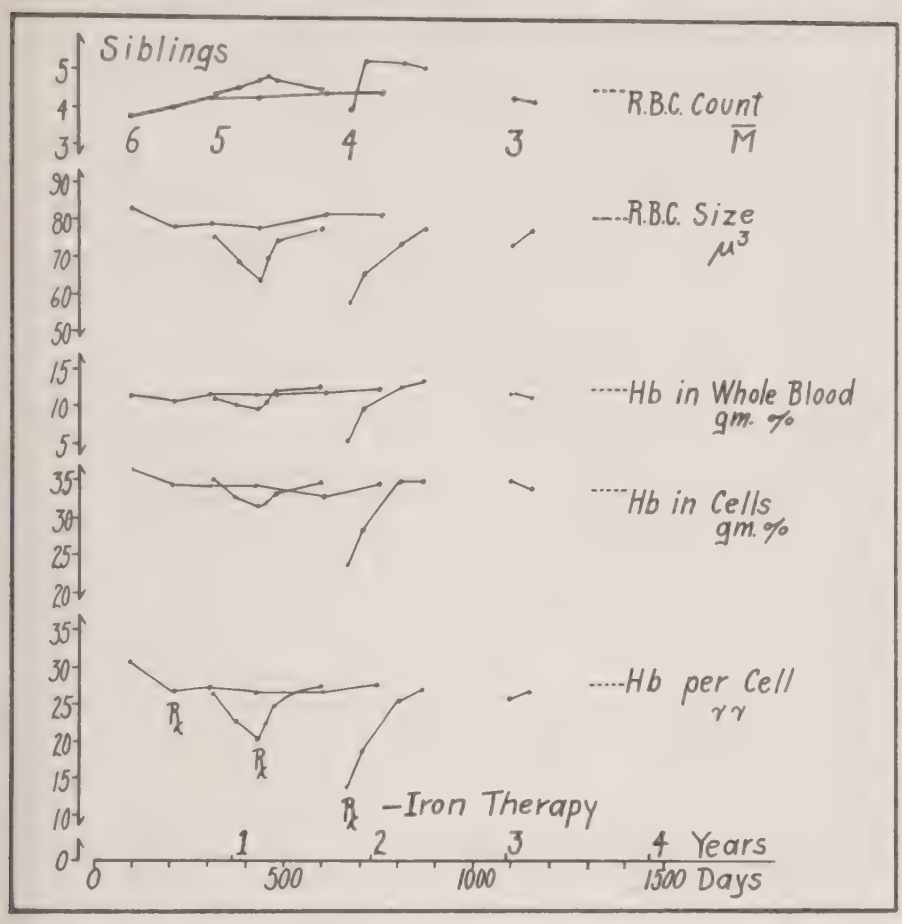



Fig. 16. Studies of a family group. The numbers, representing siblings no. 3 (male), no. 4 (male), no. 5 (female), and no. 6 (male), are placed above the first sample represented in their respective curves. The symbol ℞ indicates the time at which iron therapy was begun for siblings no. 4, 5, and 6.

family to come to our attention when, at the age of 1¾ years, he was admitted to the hospital with a respiratory infection and was found to have a severe microcytic hypochromic anemia. He was treated with iron, with excellent results shown by the rapid rise in all values to a normal level. During the same period in which he was showing his recovery, blood samples from his brother, sibling no. 3, who was 1 year older, and his sister, sibling no. 5, who was 1½ years younger, were likewise examined at intervals (without treatment). In a 90-day period the older boy showed a change in size and hemoglobin content of the cells

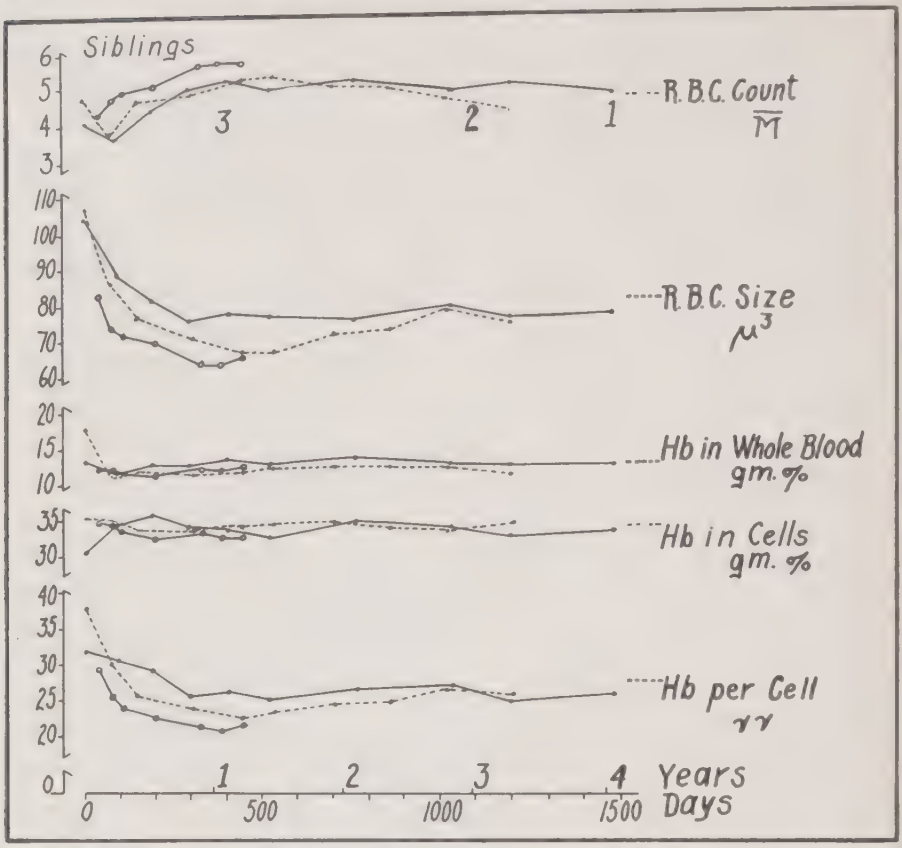



Fig. 17. Studies of a family group. Solid lines represent sibling no. 1 (female); broken lines, sibling no. 2 (female); and the solid lines with circles, sibling no. 3 (male). All appeared to have normal development, and none received iron other than that in their diets.

which suggests that these values were rising spontaneously from lower levels. Repeated samples from the girl, sibling no. 5, showed values for size and hemoglobin content of the cells decreasing at a rate from which one could predict that she, if left untreated, would develop an even more severe degree of anemia than her brother. Following iron therapy, started (indicated by the symbol) at the age of 13 months, the several values showed prompt improvement, and after arriving at expected normal levels they remained high. The sixth child, a boy born a year later, was not seen until he was 3 months old, but blood samples examined during the next 2 months showed decreasing values until the age of 5 months when he was given iron. Thereafter the values ceased to decrease, and through this second year are

remaining at a level attained by his siblings after their course of iron treatment.

In the second family (Fig. 17), sibling no. 2 was born just less than a year after sibling no. 1; and sibling no. 3, was born 2¼ years after sibling no. 2. They have had normal development and have been unusually free from colds and intercurrent infections. From this chart it is seen that there is an insignificant difference between the curves for hemoglobin in the whole blood of all three. Sibling no. 3 has had a consistently higher cell count. The curves for size and hemoglobin content of the cells show consistently lower values for the second and third siblings. The last and lowest value for size of the cells in the youngest infant is still not alarmingly low and probably it is not yet necessary to give iron. However, it may be presumed that if this mother has another child, the next infant will show earlier and greater decreases in the cell values, and should be given iron early as a prophylactic measure.

Thus our discussion of hematologic methods for detecting nutritional anemia should include also the consideration of methods of predicting the development of this condition, if we are to learn better how to prevent its occurrence. The relationship of maternal health, generally, and of the anemias of pregnancy, in particular, to the development of anemia in these infants is a most important part of this whole problem, and one that deserves much more attention from both obstetricians and pediatricians than has been given to it in the past. In the practical application of these studies, it is now a part of our teaching in the hospital and clinic that whenever the diagnosis of nutritional anemia is made, inquiry should be made about the siblings, and that iron should be given routinely to the succeeding siblings, as prophylaxis against anemia in those infants. The mother of the infant with anemia should be warned that her next infant will probably develop the same sort of anemia, and, to prevent this, administration of iron should be started early. In a succeeding pregnancy, it is wise to give such a mother iron during her pregnancy even though the hemoglobin content of her blood is not thought to be unusually low.

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## DISCUSSION

DR. FREDERIC W. SCHLUTZ: Nutritional anemia is, I believe, a more prevalent condition in the younger child, the very young and the infant, than in the adult. At least, in our clinical experience, especially in large metropolitan areas, this seems to be fairly obvious, and is a condition which we quite commonly encounter.

There are a good many conditions, I believe, which contribute to the picture besides the actual nutrition of the child. There are factors, for instance, such as environment, climate, and altitude which all enter into the picture and must be considered.

It becomes increasingly clear that there is a great deal of information to be gained in this field, and that this particular approach to this problem has large possibilities to give us not alone more accurate information than we have had heretofore, but to give us also a mechanism by which we can make very accurate appraisals of both the normal and the abnormal, and by which we may be able to predict conditions which may be in the making, and, what is equally important, by which we may verify the effects of therapy and treatment.

There are two approaches to this condition: The one Dr. Guest has described, and another one which is much more recent but which holds a great deal of interest, and also seems to have a great deal of possibility.

The one that Dr. Guest describes is purely morphological. It has this real advantage over tests of some other factors which seriously affect nutrition in that it comprises methods which in capable hands will give some really significant information.

The other approach is the chemical study in this field very recently undertaken by a group working under the Couzens Fund, of Michigan, and some work now going on in the Department of Anatomy, particularly, of the University of Chicago, dealing with the more minute chemical structure of the cell, and identification of the substances there, and their transfer or shift during various conditions of health and disease. This method is infinitely more difficult and is probably one that cannot, by its very nature, become a very practical one. It is extremely important that we have information of the normal condition that exists in the blood structure of the infant under various circumstances.

It is a fact well known, of course, that there are very significant

changes which refer to the various age periods of the child, and which definitely occur during the period of growth. They do this in the normal individual, and do it of course to a far greater degree in the organism that is suffering from disease.

What we have been lacking is a sufficient amount of data made by very carefully controlled observation, and with very accurate methods. If one looks through the literature dealing with this subject, the largest amount of effort up to this point has consisted in developing sufficiently accurate means of cell measurement and hemoglobin estimation. It has been constantly a desire to perfect instrumentation and methods that have been available but have been recognized as not being sufficiently accurate.

With these methods now becoming increasingly available and accurate, we can proceed to the type of study that Dr. Guest has undertaken on very large material. After having, then, a very reliable and dependable norm to go from, and knowing what is the picture of development that does take place from infancy to adolescence and the adult, we can compare this with the picture that exists in the various diseased conditions, particularly referable to the nutritional states or nutritional deficiences. What this will mean is that we will have a method of very accurate appraisal and a method that will enable us to classify the various forms of anemia, and also will particularly aid in their differentiation.

Dr. Glenn E. Cullen: This particular presentation emphasizes one of the main reasons for calling this group together, that is, to find the usable technics for studying abnormal nutrition. We have long had technics for determining the amount of hemoglobin and for making accurate blood studies. It is one of the tragedies of medicine that, in spite of the great amount of laboratory work that has been done in most of the clinical laboratories of the country, it has taken so long to get this information. It is amusing that it is much easier to do an accurate hemoglobin determination by means of a colorimeter than by the hit-and-miss method with older technic. It is also unfortunate that most of the older determinations have been expressed in terms which cannot be translated into modern terminology.

Dr. Mitchell, in his introductory remarks, made the point that we were looking for optimum conditions in nutrition rather than deficiencies. I think Dr. Guest's work is a very good example of the

problems facing us in deciding what is optimum. What should one use in these studies for "optimum" hemoglobin in the whole blood? If one takes the statistical manipulations and gets means or modes, then one includes all the abnormal conditions reported here. Isn't it more logical or, perhaps wiser, to use an arbitrary but reasonable upper level as the optimum condition to which we aim? If one does that, one sees Dr. Guest's point that values for infants around 2 years old, which have been accepted in the past as perhaps satisfactory (hemoglobin 10 to 11 grams per 100 cc.), are really much lower than optimum, which is probably about 12 to 13 grams per 100 cc.

In this study of nutritional anemia, we have two indices of the condition of the blood, i.e., the amount of hemoglobin and the size of cells. Attention in the recent past has been centered on iron deficiencies as evidenced by low hemoglobin levels. However, in many children in whose blood there is apparently almost enough iron, these erythrocytes are small, although increased in number. Which is the more important physiological factor? Is the compensation for decrease in size of the cells by increase in their number an evidence of normality? We are inclined to think not.

These questions emphasize the difficulty of defining "optimum." We can, at least, divorce ourselves from the danger of thinking in terms of "average values" and think in terms of zones of apparently normal or optimum conditions. The use of zones of limits of normality permits an allowance for normal individual variation which is impossible with an "average" figure.

These results emphasize another problem of infant nutrition. With reference to depletion of iron, Dr. Guest has evidence that not only is the child depleted, but that the mother is depleted and the degree of her depletion reflects itself in successive offspring. Successive siblings of a mother, especially if they are born too close together, result in successively poorer states of iron content and cell size. There is no logical reason to assume that similar depletions are not happening to other systems: to the calcium-phosphorus relations and bone development, to muscle development, and so forth.

Dr. Harold C. Stuart: I was interested in Dr. Guest's curves of individual growth. Physicians have been in the habit of taking data obtained on large groups of children of successive ages and building up what they consider to be normal growth curves for individual children. If the hemoglobin level in the blood is found to be low on

the average at 3 months of age, we say that this is normal for infants of this age. This reasoning may apply to some growth factors, but it certainly does not apply to others, and it may be that the blood is one of the exceptions. If the 3-months' infant happens to be more susceptible to conditions which lower the hemoglobin level of the blood, or if these conditions are more prevalent at this age than subsequently, then the average will be lower than should properly be considered normal.

We have been plotting the hemoglobin curves and the red counts of a group of so-called normal children that we have been following for a number of years. Even though the averages are lower at 3 months than subsequently, some individuals retain a high level at this age, and many show lower readings at 18 months than at a year, or lower at a year than at 3 or 6 months. Some individuals fluctuate tremendously from period to period, while others seem to retain very steady blood pictures, suggesting that one group is more susceptible to variation than another, or that they have had a more abnormal experience. One cannot at present speak of the average hemoglobin curve as the normal for the individual.

We have found that the hemoglobin level does seem to correlate pretty well with the adequacy of nutrition in other respects, and if a child presents a low hemoglobin reading, his diet is likely to be found poor in iron and possibly in other food essentials. From a public health standpoint, the hemoglobin level may really be quite valuable. We are quite satisfied that the clinician cannot look at a child and say he is pale or he is not; that a properly taken hemoglobin gives him information which he cannot get in any other way. I should like to urge the further use of this simple test in connection with the examination of children. It may call attention to diets that are inadequate in iron and also in other ways, particularly in the case of children who have had frequent illnesses.

Dr. Russell M. Wilder: I have two questions to ask. First, what form of iron you gave at the point where iron was prescribed? Second, what your opinion is and what the opinion of pediatricians is generally regarding the importance of the use of more copper than is normally contained as a contaminant with iron.

Dr. Harry Bakwin: Now that rickets in children has largely disappeared, anemia is, next to simple undernutrition, the deficiency

disease most frequently encountered in infants. In regard to the choice of materials for standards, Dr. Guest spoke of using a cross-section of the population. What type of child to use for a standard is a problem that you meet in every field, not simply in the choice of blood standards. It seems to me that one ought to use the best available material, and I gather that Dr. Guest feels that way, too, in his small group of optimal children.

Regarding those wide zones, i.e., the question which Dr. Cullen brought up, is: Is maximum optimum? How high ought the hemoglobin go? Is a hemoglobin of 11 as good as a hemoglobin of 13? One way, possibly, of attacking that problem is to take those children who are down below, and give them iron and see if they come up. But even that would not be an answer. The same problem obtains, of course, in determining the normal calcium and phosphorus values, and it seems to me that Dr. Cullen has brought up a very important point which relates not simply to these standards but to all standards.

Dr. George M. Guest: In connection with Dr. Schlutz's remarks concerning the value and need for more complete chemical studies of the erythrocytes, I should state that the investigations summarized here were in fact begun as a corollary to certain chemical studies of the blood cells which are still being carried on in our institution. In those studies, the values for size and hemoglobin content of the red cells have been used as helpful measures of control in the evaluation of changes in various constituents of the cells found in different pathologic conditions.

Dr. Stuart's remarks concerning the fluctuations in these values observed in individual infants emphasize the need for accurate evaluation of the effects of various factors upon the blood picture at different ages. We are, I believe, talking around the same point, namely: That a better understanding of the reasons for variability in these values in individual infants is probably more important than the statement of averages of data from large groups. The drop in cell count at 3 months which Dr. Stuart mentioned is a case in point. The greatest decreases in cell counts at this age are seen in premature infants; yet the term prematurity has only a comparative value, and similar physiological disturbances—or developmental deficiencies—may be responsible for deficient erythropoiesis (with low cell counts at this age) in infants born at full term. Likewise, in both

full-term and prematurely born infants the hypoferric anemia found in later months can be due to the same basic factors.

What is optimum? What is average? What is desirable? These questions are being debated about many problems of nutrition, and represent just one phase of the purposes of this conference. Here the questions concern each of the several characteristics of the whole blood and cells which have been discussed: the number of cells and amount of hemoglobin contained in the whole blood, and the size and hemoglobin content of the cells; all of these varying more or less independently of each other and differently at different ages.

In infants who have been allowed to develop a mild degree of anemia, under careful observation, before iron therapy was offered, the decrease in size of the red blood cells appeared to be the earliest and most important sign of the first stages of the development of anemia. Among the siblings represented in Figure 17 there appears to be no deficiency in the hemoglobin of the whole blood; yet the second and third infants each in turn show an increasing tendency to microcytosis of the erythrocytes. A question frequently asked is whether microcytosis, per se, is undesirable, when the hemoglobin of the whole blood is kept within usual "normal" limits by the compensative increase in cell count. It has been suggested that in such circumstances the development of moderate microcytosis is a normal phenomenon in body growth during this age period. The mere fact that the size of the cells as well as the hemoglobin content of the whole blood can be kept at a high level by the administration of iron (as in the case of sibling no. 6 in Figure 16) admittedly is not conclusive proof that this results in optimum conditions for growth and development. Nevertheless, the routine administration of iron to infants before the end of the first year of life appears to be increasingly favored among pediatricians, and at present the consensus among most writers on this subject is that the higher levels of hemoglobin in the blood, maintained by supplementing the usual dietary iron, are desirable.

To answer Dr. Wilder's question, we have used various preparations of iron, but mostly we are using the U.S.P. iron and ammonium citrate. It is cheap and it is effective. This, of course, contains traces of copper with other impurities, and no attempt has been made to use copper-free iron salts in these clinical studies. A few years ago we used a combination of iron and copper in many cases and for a

time were pretty well convinced that the supplement of copper was advantageous; later we obtained just as good recovery records using iron without the added copper, and now we feel skeptical concerning its value in treatment of infants. There is, of course, evidence that ferrous salts are more efficacious than the ferric salts, and we have used ferrous sulfate preparations with good results. The comparative evaluation of the efficacy of such preparations, however, has not been a part of our program.

# THE DIAGNOSIS OF NUTRITIONAL EDEMA WITH PARTICULAR REFERENCE TO THE DETERMINATION OF PLASMA PROTEINS AND CONSIDERATION OF THEIR BEHAVIOR

JOHN B. YOUMANS[1]

NUTRITIONAL edema is an edema due to a lowered plasma protein concentration and consequent decrease in colloid osmotic pressure of the blood, the reduced plasma proteins being the result of an absolute or relative deficiency of proteins in the diet or a failure to absorb the protein. This definition excludes from consideration the edema associated with various forms of renal disease in which the hypoproteinemia is due to a loss of protein (albumin) from the body. It also excludes those edemas caused by a hypoproteinemia which is the result of a failure of the mechanism for the formation of plasma proteins, such as may occur in some forms of liver disease.

Nutritional edema occurs in three general types. The first is the epidemic form such as was seen during the world war, mainly in the Central Powers but in other countries as well. The second is an endemic form, occurring in many countries, particularly in the far East. In this country, as far as I know, the only well defined locus of endemic edema is in the South. Finally, the third form is sporadic nutritional edema which is found anywhere, associated with a variety of individual causes for an inadequate diet or supply of protein including various diseases which may interfere with the intake and absorption of food.

Before leaving the definition of nutritional edema and this brief consideration of its etiology it is necessary to consider two other phases, first, the possible role of vitamins C and $B_1$ in the production of this edema and, secondly, the effect of certain secondary factors which do not conflict with the idea of a hypopro-

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teinemia as a cause of the edema but play an important part in the pathogenesis.

As to vitamins C and $B_1$ I cannot believe, at present, that either is directly concerned. The only apparent mechanism by which a deficiency in vitamin C could cause this type of edema would be by increasing capillary permeability through its action on the cement substance. The result of such an action would be an edema fluid high in protein which is not the case in what I consider to be true nutritional edema. Furthermore, in nutritional edema the diet is often adequate in vitamin C, other signs of vitamin C lack are absent and, in my experience, treatment with vitamin C has not influenced the edema.

As far as vitamin $B_1$ is concerned, I base my belief that it has no direct relation to nutritional edema largely upon the failure of vitamin $B_1$ in crystalline form to affect the disease in the majority of instances. I do not deny that there are secondary effects from vitamin $B_1$, and that following the administration of vitamin $B_1$ to some persons with nutritional edema, changes in the edema may occur. But there are several explanations for this, such as the effect upon the circulation and the heart, as Dr. Weiss has shown. Hence, I think that, at least for the purpose of this discussion, we can consider vitamin $B_1$ as having no direct effect in the production of nutritional edema, and that we are dealing with edema of a type which I have already defined.

Although a hypoproteinemia with a resulting lowered osmotic pressure is the primary cause of nutritional edema, there are a number of secondary factors which must always be kept in mind when one considers the diagnosis of nutritional edema. In the first place, there is posture. Since this is a hydrostatic type of edema which first appears in the dependent parts of the body, posture is bound to have a very important influence on its production. In particular, posture has a very important influence on its appearance at various levels of the serum proteins or the osmotic pressure as they fall below normal values, the erect posture of normal activity in an ambulatory person tending to produce edema at levels of serum proteins which would not result in edema in a recumbent patient.

A second very important factor is tissue pressure. We have made some attempts to determine exactly the influence of tissue pressure on the production of edema of this type. They have not been altogether satisfactory because of difficulties in measuring the tissue pressure. Recently we have been engaged in studying the intramuscular tissue pressure in contrast to the subcutaneous tissue pressure, and the two are very different. The pressures in the muscles rise to tremendously great heights, much greater than the pressures in the subcutaneous tissues, and we are inclined to believe that the site, perhaps the primary site, of filtration is in the muscle. At least, the greater amount of filtration occurs in the muscles, although from there the fluid may be moved to the subcutaneous tissues, which perhaps represent a sort of depot in which this fluid accumulates. In any event the change in tissue pressure as extracellular fluid accumulates is an important factor in controlling further filtration and deterring the development of edema.

A third factor is the salt and water intake. It is axiomatic that there can be no edema without fluid; and if the intake of salt and water is sufficiently restricted, the development of nutritional edema can be prevented, even in the presence of low levels of serum protein. This method of protection does not hold when carried to its ultimate—at least, not clinically—for, if the serum proteins are low enough, an edema appears even though the salt and water intake be greatly restricted. But I am not talking about that kind of patient. My discussion is aimed at the many patients with nutritional edema who do not have extremely low serum proteins.

A fourth factor is exercise, and by exercise I mean standing or walking, the ordinary exercise one takes in the performance of his duties. This varies greatly from individual to individual. I remember one patient whose nutritional edema first appeared after she had just started work as a canvasser and had walked a great deal for a few days. However, the factor of exercise works in two ways. The removal of fluid from the tissue spaces is largely a function of lymph drainage which is greatly accelerated by active muscular movements. Consequently, it is possible—in

fact, it does occur—that the individuals with nutritional edema, while they are working or walking, will wash out or pump out a good part of their edema. On the other hand, exercise, by its effect upon the blood flow, increases filtration, and as a result, when the exercise stops, there is likely to be a lag or a difference between the removal and the filtration rate, and in the interval following exercise the fluid is likely to accumulate. I might, just as an aside, say that for that reason exercise in the individual with nutritional edema who has a workable lymph drainage is a good thing to a certain extent; whereas in an individual who has any difficulty in lymph drainage, exercise is bound to exaggerate the edema. I make this point because many of the patients with chronic edema tend to develop a lymph blockage and a state of edema which then is not entirely nutritional but becomes related to the removal of fluid.

In the diagnosis of nutritional edema clinically, the first thing, of course, is the swelling. Unfortunately, we have no very accurate clinical test to determine the presence of edema. It is diagnosed when there is pitting. I say that, realizing that many pounds of water can be accumulated in the body before pitting appears. Nevertheless, from the clinical point of view, we do not make a diagnosis of edema until pitting occurs—at least, not in the sense I am talking of now. I should like to emphasize that the accumulation of water in the tissues is a dynamic thing, an inconstant thing. Water is filtered out into the tissues, and the amount is greater at one time than another. It is a physiological process and water is shifted about depending on posture, exercise, and the like. All clinicians have undoubtedly noticed the drop in weight which occurs when a patient goes to bed. If a patient is brought into the hospital and is put to bed, there is a rather constant decrease in weight. Excepting those patients with illnesses which might affect hydration, that drop in weight, I believe, is the result of the loss of what I like to call ballast water. When we stand up during the day, we must put a certain amount of water into the lower half of our bodies. When we lie down, that water leaves and distributes itself over the rest of the body, and to some extent is excreted. Of course, there is a constant

replenishment by the intake of fluid. Such considerations emphasize the variability in the interstitial water; they indicate that it is not a constant thing, and that the amount of fluid which constitutes edema cannot be sharply defined. Stages from a dry state to a state of edema occur without the possibility of specifying at exactly what point edema is present in terms of a definite amount of interstitial water.

The second point in the diagnosis of nutritional edema is the absence of other factors responsible for its occurrence. The patient has swelling. Our immediate thought is that it is cardiac or renal in origin. Ordinarily, in the absence of these two causes of edema, we next think of a nutritional edema since there are not many other common causes. We do not find many angioneurotic edemas, for example. So, in the absence of a cardiac or renal cause for the swelling, the next most likely possibility is nutritional edema.

The third step ordinarily is the determination of the serum proteins. I should like to emphasize that the lower figures in the range of serum protein values ordinarily accepted as normal are not, in my opinion, necessarily normal. I am inclined to believe that the lower values of the present normal range represent latent pathological concentrations. Certainly they are suboptimal. They have been included in the normal range because the subjects were apparently normal. However, recent studies in nutrition have emphasized the significance and importance of latent or subclinical deficiencies. Such considerations are very important in the diagnosis of nutritional edema on the basis of the serum protein concentration. Withholding a diagnosis of nutritional edema because of blood protein levels which are just within the range commonly regarded as normal fails to take into consideration the influence of other factors already mentioned—that is to say, posture, salt and water intake, exercise, and tissue pressure. At different levels of serum proteins, the influence of these factors varies greatly with regard to the production of edema. An individual whose serum proteins are just within or slightly below what we speak of as the normal range, may show under the influence of these factors operating within physiologic limits a

pathological pitting edema. I have a feeling that individuals whose serum proteins are high—I do not mean a hyperproteinemia, but a high normal—will not develop nutritional edema even under considerable stress and strain of the type I have discussed and such an individual I would consider normal. On the other hand I believe that an individual whose serum proteins are minimum, or slightly below what we ordinarily consider the normal level, will under many conditions of normal activity develop a pitting edema, which I think we can with justice call a nutritional edema. A further consideration which should not be forgotten is the fact that the development of edema with the loss of fluid into the tissues in a subject with only a slightly reduced serum concentration may in itself raise the serum protein concentration to within the so-called normal range.

Perhaps one should have put before the determination of the serum proteins, a consideration of the diet. When we have a patient with an edema, without a quite clearly apparent cause in the way of heart or renal disease, we should inquire into his diet. I have found that although one can get perhaps a rough idea of the diet from questioning, it is a method open to very great errors. In fact, I know no satisfactory way of determining directly what an individual eats unless he is under observation 24 hours a day. I have been trying to find some way to determine on large numbers of people what they ate, the protein particularly, and I know of no certain method except to station an observer with them throughout the day. I have found time after time that the individual whom I was questioning would assure me that he ate eggs, meat, and milk. When I questioned him further about the eggs, I would find that he ate one a week; that by "meat" he meant a piece of fat pork which has no more protein, or not as much, as a slice of bread; and that by "milk" he meant that he would drink milk if it was given to him, but ordinarily he did not drink it very often. So not very much dependence can be placed upon the correlation of diet as given by the patient with the occurrence of nutritional edema. Inaccuracies arise in other ways. For example, there was a diabetic girl whose diet was calculated and prescribed as sufficient, but whose need of extra protein for

growth had been neglected, and in whom a nutritional edema occurred. From this it should not be inferred that there may not be other influences in diabetes which might contribute to a nutritional edema; but when the diet was calculated, the protein was on the borderline and the deficiency was directly related to the production of her nutritional edema.

There appear to be three types of protein in the body: one, the cellular protein; another, the serum proteins; and the third, a reserve protein which can apparently be used to tide over temporary periods of lower intake. From the work of Dr. Whipple and his associates, it is known that when the serum proteins are lowered to a maintenance level, and when they have been kept at that level by an intake which is barely sufficient to maintain them, any interference with intake is reflected in a decrease in the serum proteins. Serum proteins can be replenished to some extent, probably, from the other body proteins; but at the same time they are an essential tissue which suffers as does cellular protein when the demand becomes sufficiently great, especially under conditions of lowered intake. Infections have a very definite influence on the formation of serum proteins, the destruction of serum proteins, or their absorption and utilization in the diet, so that all of these factors must be considered in the complicated cases of nutritional edema.

So far I have been talking about the endemic type of nutritional edema. In the uncomplicated form it is due solely to dietary deficiencies which are common to a group of the population or to a locality. Sporadic cases may be purely dietary, the abnormal diet due to a great variety of causes, but are most often seen in association with other diseases such as gastrointestinal disturbances, infection, tuberculosis, and diabetes, which interfere with the intake or absorption of food. In these cases the nutritional edema is a complication of the primary disease. It should also be remembered that nutritional edema may complicate or accompany other kinds of edema. It undoubtedly occurs to a considerable degree in many cases of cardiac edema, and in renal edema there is often a lowered intake of protein besides the loss of albumin in the urine.

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## DISCUSSION

Dr. John P. Peters: I think it is important in this discussion to emphasize the fact that we are interested not so much in hypoproteinemia as in hypoalbuminemia, because the two protein fractions are functionally highly differentiated. The globulin does not seem to suffer to any great extent in nutritional edema. It is unfortunate that it does not because, since albumin has the greater osmotic pressure, reduction of albumin has more effect in producing edema.

I would say in passing—and it is a dangerous thing to say because I cannot take the time to supplement my remarks—that there is increasing evidence that serum albumin serves a direct nutritive function. It is no longer necessary to believe that serum albumin must be broken down into its component amino acids before it is utilized. One of Whipple's latest articles has a bearing on this point.

One other point which I should like to make is that emphasis has been placed upon the concentration of albumin or protein in the serum alone, to the detriment of rational consideration of the problem as a whole. According to the Starling theory, serum albumin is only one of the factors involved in the production of edema or the maintenance of the normal exchange of fluids between the interstitial tissues and the blood stream. The reason that no close correlation can be found between serum protein concentrations and edema is because in a variety of conditions other factors besides the proteins are operative in the production of edema.

This is strikingly brought out in the condition of beriberi. There are, of course, many patients with reduced serum albumin as well as vitamin $B_1$ deficiency, who have beriberi. For a time I subscribed to the idea that the edema of beriberi was not related to the deficiency of vitamin $B_1$, but merely to the deficiency of serum albumin which is so commonly encountered in this disorder. This view is now quite untenable since it has been found that lack of vitamin $B_1$ gives rise to circulatory disturbances. Deficiency of protein and deficiency of vitamin $B_1$ are often associated. When edema occurs in $B_1$ deficiency with no reduction or only a slight reduction of serum protein, it must, I think, be attributed to circulatory failure of the kind which has been described by Dr. Weiss. Certainly circulatory disturbances must be placed in the list of factors which may contribute to the production of edema.

Another factor that seems to be of some importance—at least from

a statistical point of view—is anemia. Patients who have less than 50 per cent of hemoglobin will develop edema with far less reduction of serum proteins than will patients with higher hemoglobin. Although no explanation has been found for this predilection, the fact must be faced.

It is also imperative, in the consideration of this subject, to take into account the total amount of protein in the circulation, and not only the concentration found in the serum. If the Starling theory is correct all our patients with edema should react much as Weech found that his dogs reacted after plasmapheresis. As protein is withdrawn from the serum its concentration in the serum does not necessarily fall to any great extent at first because the volume of the serum diminishes with the protein, the extra fluid finding its way out into the tissues or being excreted. There is then a protein deficiency even though there is no reduction of the concentration of protein in the serum. This is only one example of a general principle that should be more frequently considered in the interpretation of concentrations of chemical components of the blood, and especially those substances which can not freely diffuse across the vessel walls.

The question of the measurement of edema is also important. I believe better methods are available than those that are in general use. In our searches for substances by which the volume of the interstitial fluids can be measured, none has been found which is ideal; but I believe better information will be obtained if more attempts are made to measure the volume of these fluids by means of thiocyanate. I can support the statement that considerable edema can accumulate before it becomes obvious in pitting. This seems to depend somewhat on the anatomical characteristics of the subject. Obese subjects may be malnourished in the sense in which Dr. Youmans used the term. Although it is difficult to demonstrate edema by physical examination in such subjects, they will lose more weight than normal persons when they are put to bed or given proper dietetic treatment.

Recent experimental studies suggest that it may be necessary to analyze the serum proteins more completely. Ordinarily these are fractionated into serum albumin and two or three types of globulin. In nephritis, when there has been extreme wastage of protein in the urine, the nature of the albumin appears to change. One might almost say—with some reservation, as yet—that it becomes an incomplete albumin. You are probably aware that Goettsch found that

when the plasma proteins are greatly depleted in nephritis the albumin loses its antigenic properties. Alving has shown that at the same time it becomes deficient of sulfur. Finally Hewitt has discovered that serum albumin can be divided into two fractions: one, a glycoprotein that is practically devoid of antigenic properties and almost free from cystine; the other a protein which can be identified with ordinary albumin, that contains almost no carbohydrate, is rich in cystine and possesses strong antigenic properties. It may be possible to link all these phenomena together as proof that when protein is wasted not only the quantity, but also the quality of the serum proteins depreciates.

If a nephritic patient wastes more than 15 grams of protein a day it has proved well nigh impossible to make him build up his serum proteins or to store any large amounts of protein in the body. It seems almost as if it were necessary for individuals to have a certain quantity of serum protein in order to maintain or replenish tissue proteins. This may be remote evidence that the serum proteins serve some direct nutritive function instead of merely floating around in the circulation to keep fluids from running out of the vessels.

Dr. L. H. Newburgh: I should like to ask: Is there a nutritional edema without low plasma proteins? As I understood Dr. Youmans, he thought there was not. I feel reasonably sure that we have seen patients in whom the plasma proteins are at the usual normal level, and who have a general edema of an origin which is not nephritic or cardiac, and whose stories were so clear that there seems to be no doubt about the nutritional nature of the edema.

One was a girl who was very short, and therefore needed a very small amount of food to keep her weight at normal, and who had overeaten and become very fat, and then put herself on a shockingly limited diet. She lost weight rapidly, and then lost her appetite, and she had gone down until finally she was said to have Simmond's disease. She came to us at that time with a marked pitting edema all over her body, with normal plasma proteins, and she weighed only 67 pounds even though she had the edema, so there is no question at all about her striking undernutrition.

Here was the edema, and here were the normal plasma proteins, and the whole condition seemed very clearly related to the strikingly poor diet. Her condition was dramatically relieved by means of vitamin B. She was given nothing else, but good food. In the course

of a week or so she began having a very striking diuresis, and lost the edema.

Another striking feature about her—and we have noticed it in several other patients—is this, that on the ordinary intake of vitamin B, or, to put it more broadly, on an ordinarily good diet—that is, the kind of diet which would be fed in the hospital on the supposition that it was normal in all regards, and certainly a diet that would for most of us be normal—she showed a tendency to reaccumulate edema. After several weeks some pitting of the lower extremities would occur. It would disappear after giving very large amounts of vitamin B, ordinary yeast. That observation was repeated until there seemed to be a distinct relationship between her unusual demand for vitamin B and the accumulation of fluid. It seemed clear that the ordinary amounts of vitamin B were not sufficient to prevent edema.

We have had another patient who exemplified the same condition, a man whose food was so simple that there was no real difficulty in deciding it was very inadequate in many respects, who had a generalized edema not due to heart or kidney disease, and who responded to a large intake of vitamin B and whose plasma proteins were normal throughout. This patient also, for reasons quite unknown to me, required very large amounts of vitamin B; whether that means poor absorption or poor something else, I do not know. But on the ordinarily good diet he would reaccumulate edema.

Accordingly, it seems to me that there are at least a few patients who develop edema in relation to poor diet, even though their plasma proteins remain normal, and that there are a few patients whose requirement for vitamin B is unusually large for causes which are quite unknown to me.

Another question which has been studied a great deal is this: How important is the loss of protein through the kidneys in the causation of low plasma proteins? In the beginning, starting with Epstein, it was taken for granted that the plasma proteins were low because so much protein ran out. There were very many attempts to increase the plasma protein by feeding large amounts, and these attempts were usually dismal failures.

There was an interesting study of this question by Bassett and Keutman at the school at Rochester, New York, who studied three typical cases of chronic kidney disease with edema and plasma proteins which were at the level of 4 per cent. These patients were first

put on a diet containing a restricted amount of protein, and it was found that there was a definite loss of protein through the kidneys, that the plasma proteins remained constant at 4 per cent for weeks, even though these patients were certainly making body protein—that is, they were synthesizing not only the protein lost through the kidneys but a considerable amount in addition to that, none of which could be shown to appear in the blood as plasma protein.

These patients were then put on a very high level of protein intake. There was a trivial but quite unimportant increase in the proteinuria, as I remember it, from 13 to 15 grams. The protein intake was increased from about 60 to 180, so that the 2 grams lost by urine could, of course, have no effect on the situation. The plasma proteins did not increase at all, even though when the intake was great there was a marked augmentation in the synthesis of protein, so that these individuals could make protein, that is, could stick together amino acids within the body in a form called protein in very large amounts, and the amount of synthesis was greatly increased by the increased intake of protein, but this process had no effect upon the level of plasma protein.

These men, of course, referred to Whipple's work. As most of you know, Whipple believes that plasma protein is made by the liver and in no other way, and that the intake of protein, as I understand it, has nothing to do with the production of plasma protein providing the intake is sufficient—that is, after you get to a certain level. if the liver is normal the plasma proteins will be made in sufficient amount and an increase in the intake will have no effect upon the level.

I have been impressed with that work. Our own experience has been exactly that of Bassett and Keutman. We have been unable to increase the plasma proteins by feeding protein. There has been no relation between the level of plasma protein and the dietary protein. So that it seems that there must be some very special mechanism for the protection of plasma proteins which, at least in the nephritics, is presumably injured, and I wonder what relation it may bear to this other question of nutritional edema. I have no information whatsoever about it.

I have been especially interested in the relation between salt and edema. It is axiomatic if we accept Starling's theory, and I think we all do, that one cannot accumulate edema without salt and water. That point does not need discussion, but I have been puzzled by

this situation: Given a patient with nephritic edema from whom the edema is removed, the reaccumulation of edema may be prevented by putting such an individual on a diet low in sodium chloride, while receiving very large amounts of water. This individual still gets sodium chloride. Why does he not slowly reaccumulate edema? Why, when he takes, we will say, 2 grams of sodium a day, does he not become edematous? But if he takes 5 or 6 grams, he will become so.

We have recently devised a very simple clinical test to decide whether an individual will become edematous. This test is confessedly clinical; it is not quantitative in the sense in which one would like to have it, but it is simple enough so that the interns can do it. One puts the patient on a diet of constant composition, the total fluid content of which is fixed—that is, the water in the food, plus the other water and liquids, is at a constant level. If the patient is then leading a constant life, if he is in bed or doing almost the same things every day, his expenditure of energy and materials will be very nearly constant, and therefore the weight of the urine plus stool will represent in a rough way a value which can be compared with the total intake of fluid which is known, because it has been prescribed, and is constant throughout the study. One can check the observation by determining the sodium in the urine and comparing it with the sodium in the diet.

Under such circumstances, a nephritic from whom the edema has been removed, and who has now reached a constant level where the output of sodium equals the intake, and who is given 10 grams of sodium chloride, will become edematous, and ordinarily about a liter of water will be added to the body. Again, in order to see whether this method in its simple form does work, we have done the sodium exchange. Such a typical nephritic will retain practically all of the added sodium during that twenty-four hours when it is given, and will retain an appropriate amount of water. During the next days there will be a very slow excretion of the sodium, so that in the course of a week or 10 days it may all have again left the body, and the extra water will come out with it. If a normal individual is treated in the same way, he will retain some of the sodium during the first 24 hours with an appropriate amount of water—perhaps half a liter of water and half the sodium. The next day it will all be eliminated.

We are impressed with the rate of elimination as the best clinical

evidence of whether the individual will easily become edematous or not. If the nephritic is given extra sodium for 3 days, he will tend to retain it all and add water appropriately, so that he may add about 3 liters of water. The normal individual, however, on the second day, will get in balance with the intake—that is, he will retain sodium and water the first day, the second day he will excrete what he takes in, hence his response is totally different. While that does not permit us to tell whether the individual has extra water in the body to start with, it does help us to decide whether the individual is normal or abnormal in regard to the question of accumulation of edema fluid. The whole point is that while it is not quantitative, it is at least a means of getting some information about the situation.

Dr. Howard W. Robinson: We have reached the stage where we should be certain that the terms "albumin" and "globulin" have the same significance to all of us. In this country the protein partition is usually determined by Howe's method, i.e., the globulin is precipitated in a 1.5 molar sodium sulfate solution at 37°, and the albumin concentration is calculated from a nitrogen determination of the filtrate. With this procedure the relationship of the albumin concentration to the globulin concentration in normal human blood serum is about 2.5 to 1. However, when ammonium sulfate is used, as it is by many French workers, the albumin-globulin ratio of normal human serum is close to unity. According to Howe, a 1.5 molar sodium sulfate solution is identical in precipitating properties with a 2 molar sodium sulfate solution, but the concentration of a half saturated solution of ammonium sulfate depends upon the temperature and is usually much higher than 2 molar.

For that reason, it is time that we standardized on one precipitating salt. This may be very empirical, but we all realize now that a single precipitation does not give a complete separation of the proteins and in most cases of clinical and public health investigations it is impractical to isolate quantitatively a number of pure protein fractions. A 1.5 molar sodium sulfate solution precipitates a complex mixture of proteins and some of the fractions may contain sugar, lipids and other nonprotein substances. However, I could present data, if time permitted, to show that this precipitation in 1.5 molar sodium sulfate solution gives reproducible separations of two fractions arbitrarily called "albumin" and "globulin."

The objections to the method have been: first, the precipitate

filters slowly so that to avoid adsorption of soluble protein by the paper the first portions filtered must be discarded; and secondly, it was thought that a 1.5 molar solution of sodium sulfate must be used at 37°. We have been able to simplify the procedure along these lines.

Some time ago we attempted to separate the precipitated globulin from the albumin by centrifuging with the large International Centrifuge. The precipitate settled very slowly and we never obtained a clear supernatant. During the last year the Swedish Angle Centrifuge, introduced by Lundgren, has been used with great success. This portable machine employs an oval tube which is revolved at an angle of 40 degrees from the vertical. With the tube in this position the precipitate travels only a short distance through the solution until it strikes the opposite side and reaches the bottom of the tube in a short time. The clear supernatant is drawn off with a pipette and a nitrogen determination is made on an aliquot. In most cases a good separation is obtained after a centrifugation of one hour. The albumin values obtained in this manner agree with those obtained by our filtering procedure.

For a long time I have often been impressed by the fact that when the 22 per cent sodium sulfate solution was removed from the 37° room, no crystallization from the solution took place. As sodium sulfate forms supersaturated solutions with ease, no thought was given to this until one day it was found that even by seeding, crystals could not be obtained. Solubility studies showed us that the solubility measurements made by Klottman were correct, i.e., 26.2 grams per 100 cc. at 25° and 18.4 grams at 20°. When we indicate "room temperature" we may mean 20°. In most laboratories, especially in the South, 25° is below the average temperature, and in the North a temperature of 25° in a room is achievable without much inconvenience. Also it is more comfortable working at 25° than at 37°. Our results, obtained from filtration or centrifugation experiments at 25°, agree with the results obtained at 37°. Therefore, the objection raised against working at 37° with sodium sulfate can be removed if the globulin separation is carried out at 25°.

The empirical nature of this separation is brought out in the solubility curves shown in Figure 1. The points on the curve represent the solubility of the serum protein expressed as the logarithm of the protein concentration in grams per liter of serum at sodium sulfate concentrations between 0.6 molar and 2.3 molar. At the two lowest

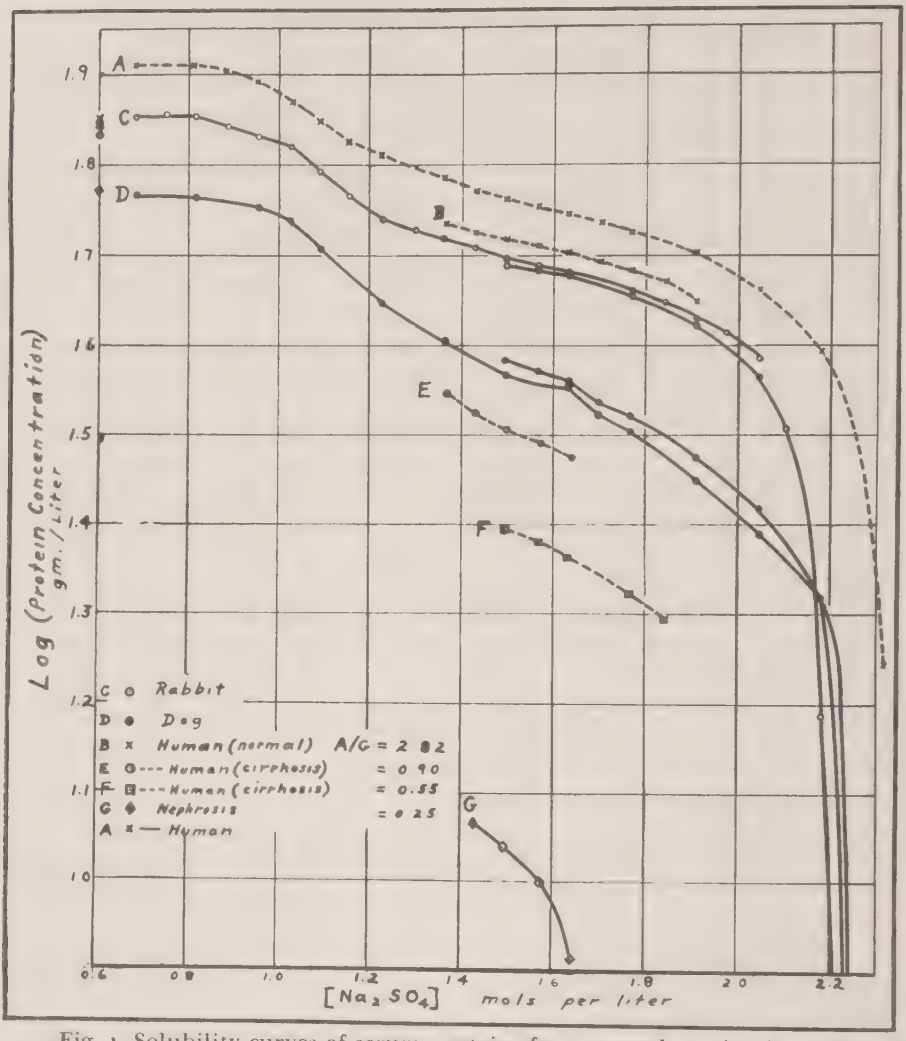


Fig. 1. Solubility curves of serum proteins from several species, including human in both normal and pathological conditions, with various concentrations of sodium sulfate.

concentrations no protein is precipitated. The curves are perfectly regular in the neighborhood of 1.5 molar and we have never obtained breaks in the curve similar to those reported by Howe. These curves show that 1.5 molar sodium sulfate is as suitable as any other concentration.

The study of these solubility curves in pathological serum has brought out some interesting points. Curve *B* (Fig. 1) represents the values obtained on a normal human serum with a total protein of 7.07 grams per 100 cc. and curve *F* represents the values obtained on a serum with a total protein of 6.95 grams from a patient with

liver cirrhosis. The albumin concentration in the normal serum is 5.2 grams per 100 cc. and in the pathological serum 2.5 grams. The latter serum has larger amounts of protein precipitated at salt concentrations between 0.8 and 1.2 molar than the normal serum and much less protein soluble above 2.0 molar. It is very interesting to us that the amount of protein precipitated *between* 1.4 and 1.9 molar in the two sera are practically the same, i.e., the kind of protein that is precipitated at these salt concentrations seems to be unaltered in this pathological state. Therefore, there is not a gradual change in solubility conditions but a change in the type of protein present. Curve *G* is that obtained on the serum from a nephrotic patient. The total serum protein concentration is 5.9 grams per 100 cc. and the albumin is 1.1 grams. This curve illustrates the type of changes that Dr. Peters has mentioned. I cannot visualize how this serum would, if the curve were continued further, have any of that type of protein precipitated only at high concentrations of salt.

Many people feel that if a foreign protein gets into the blood stream, it is excreted by the kidney without much damage to the kidney. One might speculate—and it is a wild speculation—that perhaps in a nephrotic patient the protein is modified, so it really could be considered foreign to that patient, and, therefore, it is excreted in large amounts in the urine.

There is just one other point in connection with the regeneration of serum protein. With two cases at the Children's Hospital in Cincinnati it has been interesting to note the following fact: In a patient without liver or kidney involvement whose serum protein is extremely low, that is about 3.5 grams per 100 cc., the administration of a good protein diet will raise the serum protein to 5 grams in about one week. These results are comparable to the regeneration obtained in the dog experiments of Whipple and his coworkers. But with many of the patients, who have nutritional edema with a serum protein around 5 grams, it is very difficult to build their serum protein level up to at least 6.5 grams. We feed such patients good diets for a considerable time and obtain generally a very slow response.

Dr. Harold C. Stuart: I am glad that Dr. Youmans called attention to the inaccuracy and inadequacy of the ordinary medical dietary history. He suggested that the only way to obtain a good dietary history is to have someone live with the family for a week or so and see what they eat. It has been our experience that if you ask

a person what he eats, he will give you a general answer that is far from accurate. If, however, you have a technic and your nutritionist has sufficient time to allow for a certain amount of repetition and certain checking, you can obtain a fairly accurate dietary history. Individuals in the lower economic groups tend to eat rather consistent diets, so that important deficiencies tend to be revealed by reports covering brief periods. We have found that our dietary histories, taken by a nutritionist and verified as carefully as possible, agree very well with the evidences of nutritional inadequacy revealed by such determinations as ascorbic acid and hemoglobin levels in the blood. With time and a technique which aims to detect inconsistencies in the report of the diet, it is possible to obtain what we consider a fairly accurate dietary history, at least sufficiently so to call attention to important deficiencies.

The diet history should only apply to a recent period. If you ask a patient at a maternity hospital what she ate during the first 3 months of pregnancy, you are more than likely to get a very inaccurate answer. If you ask her about her diet during the last 3 months of pregnancy, and if the nutritionist questions her according to a suitable technic, you can usually obtain a fairly accurate picture of her food habits.

Dr. John B. Youmans: It is well to emphasize what Dr. Peters emphasized, the distinction between hypoproteinemia and hypoalbuminemia. Similarly, to refer to a reversal of the albumin and globulin ratio is not a precise expression since the ratio can be reversed by greater globulin or a smaller amount of albumin. Although the reversal due to high globulin is often helpful in the diagnosis of certain conditions, it is not particularly significant in this type of edema, because the main thing is the drop in the albumin. The globulin, as a rule, stays normal; sometimes it goes down somewhat, and sometimes it is increased.

Dr. Peters also brought out the fact that these patients may have other troubles. That is true. I was referring—making the matter simple—to a group who have practically nothing else wrong with them. They come into the clinic for refraction or a bad tooth, or something of that sort. However, we do see quite a little anemia in this group, and I think it may be a factor in the edema.

Dr. Peters also spoke of a lessened blood volume which, if I understand him correctly, tends to mask a drop in the serum proteins;

there is a decrease in total protein, although not a decrease in the concentration. That can be very well shown as we did in patients whom we brought into the wards and let lie in bed for a number of days until the edema dropped to a pretty constant level. They were on a diet which approximated what they took outside. We then had them stand up and walk around the ward all afternoon, let us say. With that the edema increased and the serum proteins went up. Then we let them stay in bed again and soon they hit a constant level of edema. We then put bandages on the legs and let them stand up. No edema appeared and the serum proteins remained where they had been while the patients were in bed. That also emphasizes the importance of tissue pressure in connection with this form of edema.

We have a method of determining the tissue pressure by which we can have the patient standing and moving around a bit. We insert a needle in the muscle and connect it with a manometer to determine the tissue pressure.

Dr. Newburgh asked if cases of edema occurred without a lowering of the serum proteins. I imagine he meant cases of nutritional edema. I will have to divide the answer into two parts. I do see cases of nutritional edema with normal serum proteins, but they are chronic cases, cases in whom the tissues have been so stretched that for them it is normal (if I may use the word in quotation marks) to carry around an extra quart or so of water in each leg. Even with what we speak of as a normal serum protein, they still keep an edema.

I have not been talking about the patient with nutritional edema whom you will ordinarily see in practice in the hospital, one who comes in with an edema of the face and hands, and a sharp reduction in the serum proteins. I see them, but those are not the ones I was stressing as the endemic nutritional type. The ones I see have not complained of swelling, or very rarely do so. I think it is partly because they are so accustomed to it that they are inclined to look upon it as a more or less natural condition. Formerly—before I was where I am now—when I would ask a woman if she had swelling of the feet and she said yes, if I found an edema I was sure to find another cause such as heart or kidney trouble. In Nashville, when I asked the same question, and she said yes, she might have a pitting edema that you could shove your thumb in and yet not have heart or renal disease. That perhaps illustrates the type of patient that I was referring to. They have no complaint, or they have no typical ones, and no other important disease associated with the edema.

The second part of my answer to Dr. Newburgh applies to the type of case he reported and discussed. I cannot explain those cases. I think we must accept the fact on the basis of our present knowledge that cases of edema do occur in which vitamin $B_1$ seems to be the agent concerned without any lowering of the serum proteins. However, I have not seen such cases and know nothing about them. I think that the use of $B_1$ as a diagnostic test, counting the diuresis which occurred as an evidence of its influence, would help distinguish those cases in which $B_1$ is a factor and those in which it is not. Generally, I have not seen any particular diuresis from the administration of vitamin $B_1$, and that is one of the reasons why I think that $B_1$ is not particularly concerned with the usual type of nutritional edema.

About the injury that the edema causes, I do not know. As far as one can tell, it does not interfere except for the slight interference with function that a slightly water-logged organ or tissue might have. In the more severe states, it may be associated with bronchopneumonia.

In connection with remarks made concerning the formation of serum proteins, I may say that I have great difficulty in raising the serum protein level in chronic cases of nutritional edema. I have patients, such as others have reported, with nutritional edema, and a low serum albumin, who were treated and in whom the serum proteins increased very promptly, and they got well. I also have a group of patients who have had the disease for a long period of time, and the ones I speak of have often had it for years, in whom it is with the greatest difficulty that one can raise the serum proteins by feeding, even when he is fairly sure that they do take a pretty good diet. Dr. Robinson spoke of the difficulty in raising the serum proteins above a certain point. In a sense it is the same with these people. They hang at a slightly low level, and it is almost impossible to get them up further. Whether that has anything to do with Whipple's work—I think he intimated some difficulty in the natural formation of serum proteins after they had gotten to a low level—I do not know.

Dr. John P. Peters: I feel that the theory that the liver is the source of serum proteins is extremely dubious. Certainly to accept this theory in the sense in which Whipple first proposed it is difficult in view of the fact that serum globulin regularly increases in most

profound liver disorders. To me it does not seem logical to believe that if the liver were the source of all proteins, including globulin, the latter should increase in conditions in which the liver is most seriously damaged, such as cirrhosis.

I do not see why it should puzzle any one greatly that the serum proteins can not be built up completely in the face of a severe proteinuria, even if the patient is storing nitrogen. It is in theory, at least, possible to replenish the tissues with certain nitrogenous compounds and with protein without necessarily replenishing serum proteins. Even if the evidence that the serum proteins had a direct nutritive function were stronger, there might still be other means for the formation of tissue proteins. I stated that serum proteins could not be increased in patients who lost 15 or more grams of protein in the urine. With lesser degrees of albuminuria distinct increases of serum protein can be effected. It must be recognized that the drain on the serum proteins is more direct than the drain imposed by other forms of malnutrition. One point that has puzzled many of us, without provoking much comment, is the fact that when the serum proteins are greatly depleted by such drainage, patients waste. Most investigators who have practised plasmapheresis have found also that, when the plasma proteins are sufficiently reduced, animals lose weight even when they may be gaining edema. Apparently the nutritive state suffers some injury when the serum proteins fall to a certain level.

The effect of high water and low salt diets, of which Dr. Newburgh spoke, affords an example of the necessity of considering all biological processes as a series of linked equilibrium reactions. The administration of a small amount of salt and a large amount of water provides a very abnormal interstitial fluid, a diluted interstitial fluid, at least. Under these circumstances any kidney will react by excreting water, and with this will inevitably wash out a small amount of salt. It is reported, although I have been unable to verify it by experience, that a diuresis of a somewhat similar kind can be induced, even in severe nephritis, by the administration of large amounts of salt with very little water. I have, in some cases with depleted chlorides secured diuresis by the use of hypertonic salt solutions. To practise this procedure on the ordinary nephritic patient involves cruelty if the fluid intake is reduced to an effective degree.

The most cogent reason for feeling that sodium sulfate is the pro-

tein precipitant of choice, in my mind, is the fact that the fractionations secured by means of this salt agree with fractionations obtained by other unrelated procedures, especially the immunological and ultracentrifugal methods. Certain titration curves, particularly some that have been made with citrate, take an entirely different form from those derived with sodium sulfate. In such citrate curves from the serum of patients with nephrosis there are distinct differences of precipitability resembling those that Dr. Robinson has presented today. In his last curve, as you will see, the serum albumin fell off when there must still have been protein in the serum. From the sodium sulfate curve all the protein appears to have been precipitated, although there is still protein present. Was there more protein in that serum, by chance, than the sulfate precipitated? I should like to add to what I said about Hewitt's recent studies, that Hewitt found that when he isolated the glycoprotein from the other serum albumin fraction the glycoprotein was no longer precipitable. It was only precipitable from native serum or when it was mixed with the other albumin fraction; in this case it acted just like albumin.

Dr. Robinson mentioned that it was easier to raise the proteins from 2 to 3.5 per cent than from 5 per cent to normal. Perhaps the explanation can be found in those plasmapheresis experiments by Weech and Goettsch in which it was found that when protein is abstracted, the serum volume at first diminishes proportionately. A comparable succession of events may accompany protein regeneration. Under these circumstances concentrations of protein in the serum are no longer a good measure of the total amount of circulating protein. Nitrogen balances would be required for accurate information. It is possible, of course, that increasing resistance to regeneration of protein is only an expression of the general law of diminishing returns.

Dr. L. H. Newburgh: If it is true that one can develop edema in the presence of normal plasma proteins, and in the absence of kidney disease and heart disease, then evidently there is another important mechanism in addition to the Starling one which causes the extra accumulation of salt water in the body. I have been very much interested in that question for a long time, and it seems to me that here is some evidence that something about which we know very little needs very careful study. Evidently, edema can develop in the face of normal protein when there is too little vitamin B in the diet.

In other words, we can say in a general way the tissue is abnormal and can be made normal again by simply feeding large amounts of vitamin B.

That, to me, has always been an exceedingly interesting question about which I have no further information, except, perhaps, this: A surgeon has been greatly interested in the amount of water to supply surgical patients at different times—before operation, during operation, and immediately after—and he has divided his patients into two groups according to their response to physiological salt solution. He speaks about the "sick" surgical patients, and those "not sick." What he means, evidently, is that the sick ones are the surgical patients who have an infection; that they need an operation, but that they also have an infection. Perhaps the infection is associated with the need for the operation. Then, there is the other group in which there is no infection but an operation is needed. The sick patients, according to his definition, become edematous when they are given salt solution. The ones without the infection do not. Neither group, of course, becomes edematous when given water containing glucose. In those two groups the plasma proteins are normal; so here is another example of the extra accumulation of fluid in the body due to some fault other than the lowering of the proteins—that is, a suggestion that the state of the tissue which, of course, is a very broad term, has something to do with this whole question.

Another word or two about the matter of how much the level of plasma protein is affected by the loss of protein in the urine. In the first period of the experiments by Bassett and Keutman, which I cited, when the plasma protein level was 4, the patient was receiving 60 grams of protein and excreting 13 in the urine. In the next period, he was receiving 180 grams of protein and excreting 15. In other words, he was losing 2 more grams and getting in 120 more, so that there, at least, the failure to make plasma proteins could not have been due to extra loss because the loss was 2 and the increase 120. Now these patients, when they were getting more protein, synthesized more protein without the slightest doubt. But what they synthesized did not appear as plasma protein. That, to me, was the interesting feature about that work.

I am sure I do not know whether Whipple is correct or not. It is exceedingly difficult to get all the details of his work. I know his conviction that the plasma proteins are made in the liver, and in

the liver alone, but I am not at all sure that his experiments are conclusive because they are so difficult to follow and so difficult to carry out. Of course, we all know that a test of the state of the liver, or one of the tests, is the level of plasma protein. There does seem to be a definite relationship between the level of the protein and the state of the liver. When the liver is diseased, the plasma proteins go down as a whole.

Dr. John P. Peters: I can not see why the Starling theory need be discarded because lack of $B_1$ produces edema and administration of $B_1$ eliminates it, when it has been so clearly demonstrated that this vitamin has a striking effect on the circulation. I should be quite as willing to abandon the Starling theory because in cardiac failure or disturbances of the circulation edema occurs that responds to digitalis or to measures that support the circulation. This has been clearly brought out by Dr. Weiss' studies.

With these surgical cases I have also had some experience. When people are sick they tend to develop edema which may not be related to the serum proteins. It is essential in studying these surgical cases to find out whether in the beginning they are suffering from dehydration, which is common. Secondly, it is essential to know the kinds of fluid which are given and to learn whether normal concentrations of serum electrolytes have been established by the strange mixtures that are sometimes injected. Finally the competence of the peripheral circulation must be examined. Thus far, in a large series of cases of this kind, I have been convinced that there is no reason to abandon any part of the Starling theory. It is, however, necessary to take into consideration all dimensions of the Starling theory in the analysis of the causes of edema.

In relation to proteinuria I have extensive experiments carried on much longer than those of Bassett. The storage of nitrogen, on the whole, is not very great, while the losses of serum albumin through the urine are of the order of magnitude of the losses which Whipple was unable to replace by feeding any amount of protein in experimental animals. That is, the limit of the capacity for the reconstitution of serum albumin has been reached in these cases. In our experience, when proteinuria diminishes it is perfectly possible to reconstitute the serum proteins in all these cases.

Dr. Howard W. Robinson: I agree with Dr. Youmans that the A G ratio is less informative than values for albumin and globulin.

However, for a period of education and reorientation of clinicians in general we should continue the use of the A/G ratio in addition to stating the values for albumin and globulin. Furthermore, serum protein determinations without any additional information mean very little. It is of importance to know the condition of the patient, the therapeutic procedures used, the manner and time in which the blood sample was obtained and also the activity of the patient. Thus, the values for a total serum protein concentration and an A/G ratio figure can be interpreted in terms of protein partition. If a normal total protein concentration is obtained with a reversal of the A/G ratio, the interpretation is that the albumin must be lowered and the globulin increased; and if a total protein of about 4 grams and a reversal of the A/G ratio is obtained, it is probable that the globulin is normal or only slightly elevated and the albumin low. Many clinicians, I feel, interpret such changes satisfactorily.

Another important factor is that when the albumin-globulin ratio is reversed with a normal total protein, the prognosis is generally bad. We see that picture in liver involvements such as cirrhosis of the liver, and we also see it in multiple myelomas where the total protein may be much higher than normal. In our records there are a number of liver cases with total serum proteins that are normal or even above normal, the albumin concentration slightly reduced and the globulin concentration elevated. The decrease of the A/G ratio has been thought to serve as an index of the extent of liver failure. We have had one boy in the Children's Hospital and Convalescent Home during the last 2 years whose total protein has remained around 8 grams and the albumin has been practically constant; the values 3.8, 4.0, 3.8, 3.8, and 3.4 grams were obtained at intervals of 92, 134, 100, and 209 days. In this case there has been no change in the A/G ratio to show an increase in liver insufficiency. An increase of protein in the diet had no influence on the albumin concentration.

We have been following the course of the serum protein of a nephrotic patient since 1935. Two years ago the serum albumin was down to 1.7 grams and at that time the patient was losing between 15 and 20 grams of protein in the urine. This patient's serum has never shown an elevation of the nonprotein nitrogen. During the last 2 years the patient has not been losing more than 2 or 4 grams of urinary protein a day. However, the rise in the serum protein level has been very slow. In 1 year the serum albumin increased from only

1.7 grams to 3.8, and 16 months later it had reached a normal value of 5 grams. I think that has been our experience with a number of patients. When the patient is losing large amounts of protein in the urine, it is difficult to build up the protein level to its normal value; and after the urinary protein loss has diminished, it takes time to restore the serum protein concentration.

# APPENDIX

# MILBANK MEMORIAL FUND

## Board of Directors, Officers and Technical Board

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# PARTICIPANTS IN THE ROUND TABLE ON NUTRITION AND PUBLIC HEALTH

## SIXTEENTH ANNUAL CONFERENCE OF THE MILBANK MEMORIAL FUND · MARCH 29-31, 1938

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